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September 24, 1992

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Attn: Section 8(e) Coordinator (CAP Agreement)

Re: CAP Agreement Identification No. 8ECAP-0110

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith submits the following reports pursuant to the terms of the TSCA §8(e) Compliance Audit Program and Union Carbide's CAP Agreement dated August 14, 1991 (8ECAP-0110). These reports describe teratology studies with ethylene glycol monethyl ether acetate (CASRN 111-15-9).

- (1) "Ethylene Glycol Monoethyl Ether Acetate (EEAc): Probe Inhalation Teratology Study in Rabbits", ICI (UK), Report No. CTL/T/2043, April 22, 1983.
- (2) "Ethylene Glycol Monethyl Ether Acetate (EEAc): Inhalation Teratology Study in Rabbits", ICI (UK), Report No. CTL/P/840, August 25, 1983.

Complete summaries of these reports are attached.

Previous TSCA Section 8(e) or "FYI" Submission(s) related to this substance are:

(None)

Previous PMN submissions related to this substance are: (None)

cttp840


0/9/95

(2)

This information is submitted in light of EPA's current guidance. Union Carbide does not necessarily agree that this information reasonably supports the conclusion that the subject chemical presents a substantial risk of injury to health or the environment.

In the attached reports the term "CONFIDENTIAL" may appear. This precautionary statement was for internal use at the time of issuance of these reports. Confidentiality is hereby waived for purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that the publication rights to the contained information are the property of Union Carbide.

Yours truly,



William C. Kuryla, Ph.D.
Associate Director
Product Safety
(203/794-5230)

WCK/cr

Attachment (3 copies of cover letter, summaries, and reports)

SUMMARY

3

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY B REPORT (CONFIDENTIAL)
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Sponsor: Chemical Manufacturers*
Association Washington
DC 20037
CTL Ref: Y01321/002
Study No: RB0222
Copy No: 22

B-23

→ REPORT NO: CTL/P/840

ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE (EEAc): INHALATION TERATOGENICITY
STUDY IN RABBITS

by

D J Tinston
J E Doe
M Killick
M Thomas

* This work was carried out for the Glycol Ethers Program Panel of the
C M A under Contract No. GE -11.0-Ter-Ihl-ICI

Approved for Issue: *[Signature]*

Date of Issue: 25 AUG 1983

SUMMARY

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2.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc): INHALATION TERATOGENICITY STUDY IN RABBITS

SUMMARY

Groups of pregnant Dutch rabbits were exposed to 0 (Controls), 25, 100 or 400ppm of EEA_c for 6 hours a day on days 6-18 (inclusive) of gestation. On day 29 of gestation, the rabbits were killed and their ovaries and uterine contents were examined.

Exposure to 400ppm EEA_c caused reduced maternal weight gain and food consumption and also reduced the blood haemoglobin level but there were no clinical abnormalities which could be attributed to treatment. There were no effects upon the dams at 100ppm or 25ppm EEA_c.

There was an increase in the number of rabbits with total resorptions at 400ppm EEA_c and a reduction in the weights of the surviving foetuses. There was also a reduction in foetal weight at 100ppm EEA_c, which may be associated in part with increased litter size at this concentration. Exposure to 25ppm EEA_c had no effect on foetal numbers or foetal weight.

In the foetuses from the rabbits exposed to 400ppm EEA_c there was retarded ossification indicative of foetotoxicity. There was slight foetotoxicity at 100ppm EEA_c in the form of retarded ossification, but there was no evidence of foetotoxicity at 25ppm EEA_c.

At 400ppm EEA_c there was some evidence of teratogenicity as shown by major malformations of the vertebral column. There was no evidence of teratogenicity at either 100ppm EEA_c or 25ppm EEA_c.

SUMMARY

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY B REPORT (CONFIDENTIAL)
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Sponsor: Glycol Ether Program Panel*
Chemical Manufacturers
Association Washington
CTL Ref: Y01321/002
Study No: R80220
Copy No: 18

REPORT NO: CTL/T/2043

ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE (EEAc): PROBE INHALATION
TERATOGENICITY STUDY IN RABBITS

by

D J Tinston

by Moran
Ref. No. GE-176
Date 4-20-83

* This work was carried out for the Glycol Ethers Program Panel of the
C M A under Contract No. GE -11.0-Ter-Ih1-ICI.

Approved for Issue: J E Doe

Date of Issue: 22 APR 1983

SUMMARY

6

2.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc): PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

SUMMARY

Pregnant Dutch rabbits were exposed by inhalation to 450, 250, 100 or 0 ppm of EEAc in air for 6 hours per day on days 6 to 18 (inclusive) of gestation.

Evidence of maternal toxicity (reduced bodyweight gain and food consumption) in the first few days of exposure were seen at all three exposure levels. At the post mortem examinations on day 21 of gestation evidence of foetotoxicity (reduced foetal weights) was seen at all three exposure levels and in addition, there was an increase in the incidence of intra-uterine deaths at 450ppm.

There were no changes in clinical condition, haematological parameters or spleen weights in any of the EEAc exposed groups, and there were no macroscopic pathological abnormalities or external foetal abnormalities which could be attributed to exposure to EEAc.

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY B REPORT (CONFIDENTIAL)
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Sponsor: Glycol Ether Program Panel*
Chemical Manufacturers
Association Washington
CTL Ref: Y01321/002
Study No: RB0220
Copy No: 18

B-22

REPORT NO: CTL/T/2043

ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE (EEAc): PROBE INHALATION
TERATOGENICITY STUDY IN RABBITS

by

D J Tinston

RECEIVED BY CMA
SPONSORING ORGANIZATION
From: Moran
Ref. No. GE-176
Date 4-26-83

* This work was carried out for the Glycol Ethers Program Panel of the
C M A under Contract No. GE -11.0-Ter-Ihl-ICI.

Approved for Issue: J E Doe



Date of Issue: 22 APR 1983

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

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ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

We, the undersigned, declare that this report constitutes a true record
of the actions taken and the results obtained in the above study.

J E Doe

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IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY B REPORT (CONFIDENTIAL)
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Sponsor: Glycol Ether Program Panel*
Chemical Manufacturers
Association Washington
CTL Ref: Y01321/002
Study No: RB0220
Copy No: 18

REPORT NO: CTL/T/2043

ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE (EEAc): PROBE INHALATION
TERATOGENICITY STUDY IN RABBITS

by

D J Tinston

For the use of the CMA
Sponsor's Representative

From Moran
Ref. No. GE-174
Date 4-26-83

* This work was carried out for the Glycol Ethers Program Panel of the
C M A under Contract No. GE -11.0-Ter-Ihl-ICI.

Approved for Issue: J E Doe



Date of Issue: 22 APR 1983

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

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ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

SUMMARY

Pregnant Dutch rabbits were exposed by inhalation to 450, 250, 100 or 0ppm of EEAc in air for 6 hours per day on days 6 to 18 (inclusive) of gestation.

Evidence of maternal toxicity (reduced bodyweight gain and food consumption) in the first few days of exposure were seen at all three exposure levels. At the post mortem examinations on day 21 of gestation evidence of foetotoxicity (reduced foetal weights) was seen at all three exposure levels and in addition, there was an increase in the incidence of intra-uterine deaths at 450ppm.

There were no changes in clinical condition, haematological parameters or spleen weights in any of the EEAc exposed groups, and there were no macroscopic pathological abnormalities or external foetal abnormalities which could be attributed to exposure to EEAc.

1. INTRODUCTION

Ethylene glycol monoethyl ether acetate (EEAc, 2-ethoxyethyl acetate) is a solvent for nitrocellulose, low viscosity cellulose acetate and resins. EEAac has a boiling point of 156°C at 760mm Hg and a vapour pressure of 2mm Hg at 20°C. Inhalation could, therefore, be a route of exposure for users of this material.

Truhaut et al (1979) reported that rats and rabbits exhibited transient haematuria when exposed to the saturated vapour of EEAac for four hours. However, haematuria, ketonuria, haematological changes and bodyweight effects were not seen in rats and rabbits exposed to 200ppm of EEAac, four hours/day, five days/week for ten months.

Nagano et al (1979) reported testicular atrophy and leukopenia in mice gavaged with EEAac five days/week for five weeks; doses of at least 1000mg/kg/day were required to produce these effects. These authors also reported similar effects with ethylene glycol monoethyl ether which has been shown to be embryo-toxic and teratogenic in rats at 202ppm (Andrew et al 1981).

It was, therefore, appropriate to investigate the teratogenicity of EEAac. The study described in this report was designed to assess the effects of inhalation of EEAac on maternal toxicity, embryo/foetal toxicity and mortality in pregnant rabbits in order to assign suitable exposure levels for a subsequent teratology study.

Exposures to EEAac started on 6 June 1982 and the final post mortem examinations were conducted on 24 June 1982.

All original data relating to this study are maintained in the Archives of Central Toxicology Laboratory and copies of this report are held by the Reports Centre, Central Toxicology Laboratory.

2. EXPERIMENTAL PROCEDURES

2.1 Test Material

EEAc was assigned a Central Toxicology Laboratory reference number of Y01321/002. The test material (99% pure) was supplied by Imperial Chemical Industries PLC, Petrochemicals and Plastics Division, Wilton, Middlesbrough, UK, along with analytical details. (Appendix 1).

2.2 Animals and Husbandry

Virgin, female, Dutch rabbits (5-7 months old, 1.4 - 3.1kg on day of mating) supplied by Goreside Rabbits, Northchurch, Berkhamsted, UK were used. They were delivered to the experimental unit where they were housed individually in cages (47 x 45 x 58cm) in a mobile rabbit unit. Each rabbit was identified by a numbered metal ear tag. They were acclimatised to the exposure chambers (see 2.5), where they were individually housed in mesh cages (45 x 21 x 20cm), for 4 days prior to exposure for no more than 6 hours/day. Six bucks of proven fertility supplied by the same breeder were housed within the experimental unit. Food (CRB pellets) supplied by Labsure Animal Diets, Poole, Dorset, UK (Appendix 2) and tap water, via an automatic drinker system were available for all animals ad libitum except when housed in exposure chambers.

2.3 Experimental Design

Thirty-two does were mated and allocated to the four experimental groups shown in Table 1. On each day of mating, the first four does mated were allocated to Groups 1 - 4. This procedure was followed until there were two mated does per group on each day of mating.

Table 1

Group	Exposure Conc ⁿ of EEAc ppm	Experimental animal numbers
1	0(Controls)	1 - 8
2	100	9 -16 ^a
3	250	17 -24 ^a
4	450	25 -32

^a Does 14 and 19 aborted shortly after mating. The size of the fetuses indicated that the does must have been pregnant before delivery. These two does were therefore excluded from the study.

2.4 Mating

Each doe was placed with a buck and coitus observed. The fertility of each buck was confirmed by taking a vaginal smear from the first doe each buck mated with and examining the smear for the presence of live sperm. After mating each doe received an intravenous injection of 25IU of chorionic gonadotrophin ('PREGNYL', Organon Laboratories Limited, Morden, Surrey, UK) to promote ovulation. The mating took place on four consecutive days; eight does on each day. The day of mating was termed Day 0 of pregnancy.

2.5 Exposure Chambers (Figure 1)

On days 6-18 (inclusive) of gestation the rabbits in groups 2, 3 and 4 were exposed to the appropriate concentration of EEAc for 6 hours/day in exposure chambers (Doe and Tinston, 1981). The rabbits in group 1 were also placed in chambers of the same design but they were exposed to air only.

The chambers had an internal volume of approximately 3.4m^3 . They were constructed of stainless steel and access was gained to each chamber through a door fitted with a safety glass window. Air entered at the front of each chamber and was extracted at the back. Within each chamber were six cage levels and excreta collection trays which rotated concurrently (0.5 times per minute) with the direction of flow of the input air. The air flow rate was set to 600 l/min approximately using a flowmeter (ROTAMETER).

The chamber air supply was conditioned nominally to 21°C and 50% relative humidity. The temperature and relative humidity in each chamber were recorded daily during each exposure period (Appendix 3).

2.6 Atmosphere Generation and Analysis

Dynamic atmospheres of EEAc vapour were generated by metering appropriate amounts of vapourised EEAc into the input air of each chamber. The method of generation of EEAc vapour is detailed in Appendix 4.

The concentration of EEAc in each chamber was analysed between four and twelve times per exposure period. Each of the chambers had a sampling point in the back wall connected by 4mm i.d. copper tubing to an infra-red analyser (WILKS-MIRAN). The infra-red analyser was used with path length 8.5m, wavelength $8.5\mu\text{m}$ and slit width 1mm. Calibration was achieved by injecting measured volumes of EEAc liquid into the analyser in the closed-loop mode.

The rate of use of the test material was recorded in order to make a comparison with the analysed concentrations and the target concentrations.

2.7 Clinical Observations, Bodyweights and Food Consumption

The clinical condition of all animals was recorded daily and they were observed during each exposure period for any signs of abnormal behaviour.

The bodyweight of each rabbit was recorded on days 0, 5, 6, 10, 14, 18 and 20 of pregnancy.

The amount of food consumed by each rabbit was measured by offering each rabbit a weighed amount of food on days 0,6,10,14 and 19 of gestation. The residue was then weighed on days 6,10,14,19 and 21 of gestation.

2.8 Terminal Investigations

All rabbits were killed with an overdose of halothane B.P. (FLUOTHANE, Imperial Chemical Industries PLC) and subjected to a post mortem examination. One rabbit (number 29, 450ppm group) was killed on day 16 of pregnancy since it was found to be moribund (see 3.2).

The other rabbits were killed on day 21 of pregnancy.

Blood samples were taken by cardiac puncture and placed in pots containing ethylenediaminetetraacetic acid (EDTA). The following assays were carried out using a Coulter 'Model S': haemoglobin, haematocrit, total white cell count, red cell count, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration.

Spleen was weighed and stored with thymus and abnormal tissues in formal saline but the tissues were not subsequently examined microscopically.

The uterus was dissected out and the gravid uterus was weighed. The number of corpora lutea in each ovary was counted. The uterus was opened by an incision on the abdominal wall and the numbers of live foetuses, early intra-uterine deaths and late intra-uterine deaths were counted. Intra-uterine deaths were identified as being late when embryonic or foetal tissues in addition to placental tissue were distinguishable. Each live foetus was then removed from its uterine attachment and freed of foetal membranes. The umbilical cord was severed and the foetus was weighed. Each foetus was examined externally for gross abnormalities including cleft palate. All foetuses were then killed by cervical dislocation and discarded.

2.9 Statistical Analysis

Statistical analyses were carried out by comparing the results of each group exposed to EEAc with the results of the control group. The following parameters were compared by Student's t-test: maternal bodyweight gain, food consumption, spleen weights, haematological parameters, gravid uterus weight, foetal weight (calculated from the mean live foetal weight per litter), number of implantations, number of live foetuses, and number of early and late intra-uterine deaths.

Pre-and post-implantation losses per litter were calculated from the following equations:

% pre-implantation loss=

$$\frac{(\text{number of corpora lutea} - \text{number of implantations}) \times 100}{\text{number of corpora lutea}}$$

% post-implantation loss=

$$\frac{(\text{number of implantations} - \text{number of live foetuses}) \times 100}{\text{number of implantations}}$$

Pre-and post-implantation losses per litter were subjected to arcsine transformation (Fisher, 1954) and the group mean values compared using Student's t-test; the proportion of females affected per group were compared using Fisher's exact test.

3. RESULTS

3.1 Atmosphere Analysis (Tables 2 and 3)

The daily mean analysed concentrations of EEAc were within $\pm 10\%$ of the target levels apart from one day when the mean analysed concentration of the 100ppm level was 89ppm (-11%), and one day when the 250ppm level was 216ppm (-14%) (Table 2).

Nominal concentrations calculated from the rate of use of EEAc liquid are shown in Table 3. The overall variations from the analysed concentrations were +14% (100ppm level), -10% (250ppm level) and -8% (450ppm level) which were considered to be acceptable. Thus the analysed concentrations and the nominal concentrations indicate that the rabbits in each group were exposed to concentrations of EEAc which were close to the target levels.

3.2 Clinical Observations

Blood was seen on the excreta collection trays of three rabbits (number 6 - group 1 on day 17, number 12 - group 2 on day 19, and number 30 - group 4 on days 18, 19 and 20 of gestation). Numbers 6 and 30 were found to have 100% post-implantation loss at autopsy and evidence of abortion was found at autopsy of number 12. One other rabbit (number 16 - group 2) aborted but evidence for this was only found at autopsy. On day 16 of gestation rabbit number 29 (group 4) was found to have marked ataxia, loss of withdrawal reflex, and slight head tremors. This rabbit was immediately sacrificed and subjected to a post mortem examination. 100% post-implantation loss was found but there were no indications of the cause of the clinical signs.

There were no other clinical abnormalities during or after exposure or on non-exposure days which could be attributed to exposure to EEAc.

3.3 Bodyweight Gains (Table 4)

Bodyweight data from non-pregnant rabbits, rabbits with abortions, and the one sick rabbit autopsied on day 16 were excluded. The mean bodyweight gains of the three groups of rabbits exposed to EEAc were lower than the controls during the first few days of exposure. The effect appeared to be related to exposure concentration and statistical significance was achieved in the 450ppm group. Subsequently, there was a recovery in the bodyweight gains of the EEAc exposed rabbits, although the overall bodyweight gain of the 450ppm group was slightly less than the controls. The low value for the control group on days 10-18 was due to the weight loss of one animal which was found to have totally resorbed fetuses at autopsy.

3.4 Food Consumption (Table 5)

Food consumption data from non-pregnant rabbits, rabbits with abortions, and the one sick rabbit autopsied on day 16 were excluded. The mean food consumption values of the three groups of rabbits exposed to EEAc were lower than the controls during the first few days of exposure and the effect was statistically significant in the 450ppm group. Subsequently, the food consumed by the EEAc exposed rabbits was higher than the controls and during the post-exposure period, the food consumed by the 450ppm group was statistically significantly higher than the controls.

3.5 Terminal Investigations

3.5.1 Haematology (Table 6): There were no changes in any of the haematological parameters which could be attributed to exposure to EEAc.

3.5.2 Spleen Weights (Table 7): The group mean spleen weights of the rabbits exposed to 450ppm of EEAc were slightly lower than the controls but the values for the 100 and 250ppm groups were higher than the controls. None of these differences were statistically significant and there was therefore, no evidence for an effect on spleen weights.

3.5.3 Maternal Macroscopic Observations (Table 8): There was no evidence that exposure to EEAc affected the incidence of macroscopic observations.

3.5.4 Litter Data (Table 9): Data from rabbits with abortions and the one sick rabbit autopsied on day 16 were excluded. The group mean percentage pre-implantation losses and the percentage of litters with any pre-implantation loss of all three groups exposed to EEAc were higher than the controls. The differences were statistically significant at 100 and 450ppm, but not at 250ppm.

There was a dose-related increase in the percentage of litters with any post-implantation loss at all three exposure levels of EEAc, but the group mean percentage post-implantation loss was only increased in the

450ppm group compared with controls. None of these increases in post-implantation loss were statistically significantly different from controls. The only statistically significant increase in intra-uterine deaths was in the 450ppm group where the mean number of early deaths was higher than in the controls.

The mean number of live foetuses per litter was only reduced in the 450ppm group although this reduction was not statistically significant. There was a dose-related and statistically significant reduction in the mean foetal weights of all the EEAc exposed groups accompanied by reductions in mean gravid uterine weights.

3.5.5 External Foetal Abnormalities: The only external foetal abnormalities were in two foetuses from different litters in the control group which appeared pale, and one foetus in the 450ppm group which appeared oedematous.

4. DISCUSSION

Pregnant Dutch rabbits exposed by inhalation to 100, 250 and 450ppm of EEAc for 6 hours per day on days 6 to 18 of gestation had reduced bodyweight gains and food consumption values compared with controls. These effects were confined to the first few days of exposure and subsequently the bodyweight gains and food consumption values were similar to, or better than, the control values. Although these changes are indicative of maternal toxicity there were no exposure-related effects on spleen weights, haematology or the incidence of maternal macroscopic abnormalities.

The increased incidence of pre-implantation loss observed at all three exposure levels of EEAc may be the result of a non-specific effect on implantation which occurs at about the time of the first exposure period (day 6 of gestation).

Evidence of foetotoxicity was observed at 100, 250 and 450ppm since there was a dose-related decrease in mean foetal weights per litter associated with decreased mean gravid uterine weights. A statistically significant increase in early intra-uterine deaths was observed in the 450ppm group only and this was associated with higher post-implantation loss compared with controls. At 100 and 250ppm there was a dose-related increase in the percentage of litters with any post-implantation loss, but the mean percentage post-implantation losses were lower than controls and the mean numbers of live foetuses per litter were not reduced. Thus, EEAc at 450ppm caused an increased incidence of intra-uterine deaths and a depression of foetal growth whilst at 250 and 100ppm foetal growth depression only occurred.

5. CONCLUSIONS

When pregnant Dutch rabbits were exposed by inhalation to 100, 250 and 450ppm of EEAc for 6 hours per day on days 6 to 18 of gestation, maternal toxicity (reduced body weight gain and food consumption) and foetotoxicity (reduced foetal weights) were observed at all three exposure levels. An increase in the incidence of intra-uterine deaths was observed at 450ppm only.

DJT/SHR (224)

12.04.83

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ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 2

DAILY MEAN CHAMBER CONCENTRATIONS

Date	Target Concentration 100ppm	Target Concentration 250ppm	Target Concentration 450ppm
6.6.82	90 (4) 6	232 (5) 50	419 (5) 60
7.6.82	89 (8) 9	247 (7) 33	493 (9) 50
8.6.82	92 (8)12	249 (8) 23	464 (8) 32
9.6.82	93 (7)12	216 (7) 14	470 (8) 81
10.6.82	97 (7) 8	246 (7) 32	472 (8) 56
11.6.82	101 (8) 4	235 (8) 15	462 (8) 46
12.6.82	101 (9) 4	237 (9) 29	445 (10) 43
13.6.82	101 (10) 5	244 (10) 37	456 (10) 47

Values are means \pm standard deviation with numbers of analyses in parentheses.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 2 - continued

DAILY MEAN CHAMBER CONCENTRATIONS

Date	Target .. Concentration 100ppm	Target Concentration 250ppm	Target Concentration 450ppm
14.6.82	100 (10) 6	230 (10) 61	455 (10) 69
15.6.82	100 (9) 8	267 (10) 33	455 (10) 48
16.6.82	97 (10) 7	264 (10) 44	454 (10) 92
17.6.82	99 (10) 9	264 (10) 32	447 (11) 42
18.6.82	95 (10) 9	268 (11) 35	467 (11) 48
19.6.82	100 (11) 8	251 (11) 22	448 (12) 37
20.6.82	97 (11) 7	260 (11) 18	452 (12) 33
21.6.82	100 (10) 6	259 (10) 11	445 (10) 34
Overall	97 (16) 4	248 (16) 15	457 (16) 16

Values are means \pm standard deviation with numbers of analyses in parentheses.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 3

DAILY NOMINAL CHAMBER CONCENTRATIONS OF EEAc(ppm)

Date	Target Concentration of EEAc(ppm)		
	100	250	450
6.6.82	-	-	-
7.6.82	95	277	427
8.6.82	104	235	417
9.6.82	112	207	421
10.6.82	114	221	425
11.6.82	114	225	400
12.6.82	111	202	416
13.6.82	109	204	400
14.6.82	110	233	407
15.6.82	112	229	436
16.6.82	110	216	447
17.6.82	113	217	411
18.6.82	113	227	439
19.6.82	120	215	426
20.6.82	112	218	406
21.6.82	118	230	423
Overall mean	111	224	420
± SD	6	18	14
Overall mean nominal/ Overall mean analysed	1.14	0.90	0.92
Overall mean nominal/ target	1.11	0.90	0.93

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 4

GROUP MEAN BODYWEIGHT GAINS

Exposure concn of EEAe (ppm)	Bodyweight (g) on day 0	Bodyweight gain (g) between gestation day numbers:				
		0-5	5-10	10-18	18-20	0-20
0 (Control)	2079 ±108 (5)	43.8 ±72.4 (5)	23.8 ±27.3 (5)	-16.8 ±126.2 (5)	52.8 ±36.8 (5)	103.6 ±152.2 (5)
100	2130 ±401 (5)	9.0 ±77.2 (5)	0.0 ±77.6 (5)	61.8 ±50.8 (5)	46.8 ±23.2 (5)	117.6 ±208.4 (5)
250	2285 ±396 (7)	33.1 ±114.5 (7)	-46.6 ±90.5 (7)	64.9 ±39.4 (7)	89.3 ±60.8 (7)	140.7 ±158.6 (7)
450	2162 ±258 (6)	24.0 ±112.6 (6)	-115.2** ±71.5 (6)	81.8 ±33.6 (6)	98.3 ±31.4 (6)	89.0 ±110.3 (6)

Values are means ± standard deviation with numbers of animals in parentheses - non-pregnant animals and animals which aborted were omitted. One sick animal was also omitted.

** Statistically significantly different from the control group mean at the 1% level (t-test, two-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 5

GROUP MEAN FOOD CONSUMPTION (G/ANIMAL/DAY)

Exposure concn of EEAe (ppm)	Food Consumption on Gestation Days			
	0-6	6-14	14-19	19-21
0 (Control)	83.0 ±23.8 (5)	82.6 ±11.0 (5)	66.0 ±48.2 (5)	91.6 ±46.2 (5)
100	80.4 ±22.2 (5)	66.4 ±37.0 (5)	74.4 ±41.3 (5)	92.4 ±40.3 (5)
250	83.9 ±32.1 (7)	72.6 ±43.7 (7)	75.6 ±29.8 (7)	129.4 ±31.0 (7)
450	98.3 ±34.8 (6)	44.8* ±25.2 (6)	78.7 ±21.3 (6)	145.7* ±19.3 (6)

Values are means ± standard deviation with numbers of animals in parentheses - non-pregnant animals and animals which aborted were omitted. One sick animal was also omitted.

* Statistically significantly different from the control group mean at the 5% level (t-test, two-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 6

HAEMATOLOGICAL PARAMETERS

Exposure concn of EEAc(ppm)	Hb g/dl	Hct	RBC $\times 10^{12}/l$	MCV fl	MCH pg	MCHC g/dl	WBC $\times 10^9/l$
0 (Control)	12.2 ± 1.0 (7)	0.371 ± 0.032 (7)	5.43 ± 0.44 (7)	70 ± 4 (7)	22.3 ± 0.5 (7)	32.9 ± 1.4 (7)	4.5 ± 3.3 (7)
100	11.9 ± 1.2 (6)	0.365 ± 0.031 (6)	5.41 ± 0.59 (6)	69 ± 2 (6)	21.9 ± 0.5 (6)	32.5 ± 0.7 (6)	5.0 ± 2.0 (6)
250	11.4 ± 1.4 (7)	0.347 ± 0.040 (7)	5.13 ± 0.89 (7)	70 ± 4 (7)	22.4 ± 1.4 (7)	32.8 ± 0.7 (7)	4.0 ± 2.1 (7)
450	11.9 ± 0.9 (6)	0.356 ± 0.025 (6)	5.47 ± 0.52 (6)	67 ± 2 (6)	21.6 ± 1.0 (6)	33.2 ± 0.7 (6)	3.8 ± 2.1 (6)

Values are means \pm standard deviation with numbers of animals in parentheses.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 7

GROUP MEAN SPLEEN WEIGHTS (g)

Exposure concentration of EEAc (ppm)			
0	100	250	450
1.26	1.58	1.41	1.06
±0.51	±0.74	±0.51	±0.31
(8)	(7)	(7)	(7)

Values are means ± standard deviation with number of animals in parentheses.

One sick animal in the 450ppm group was excluded.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 8

MATERNAL MACROSCOPIC OBSERVATIONS

		Exposure concentration of EEAe (ppm)		
		0 (control)	100	250
				450
F7: Diaphragmatic hernia. Liver small F2,8: Greyish white patches in liver F5: Surface of kidneys pitted F6: Accentuated lobulation of kidneys F4: Two secondary spleens present.	F16: Yellow mass in thorax.	F24: Ascites	F32: Accentuated lobulation of liver	
	F9,15: Surface of kidneys pitted	F23: Rectum distended with gas	F31: Surface of kidneys pitted.	
	F10: Blood in uterus external to amniotic sac.	F21: Surface of kidneys pitted.	F30: Thymic lymph node enlarged.	
	Surface of kidneys pitted Greyish white patches in liver	F22: Accentuated lobulation of liver	F29: Red/brown mucoid fluid in uterus. Adipose tissue firm/friable.	
	F11: Fluid in thorax. F12: Surface of kidneys pitted. Accentuated lobulation of liver.	F18: Blood stained fluid in thorax Patchy congestion of lungs. Surface of kidneys pitted.	F25: Surface of kidneys pitted. Accentuated lobulation of liver	
			F28: Dark red spots on lungs. Accentuated lobulation of liver.	
Total no of observations	7	9	7	9
No of does affected	6	6	5	6
No of does autopsied	8	7	7	8

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 9

LITTER DATA

	Exposure concentration of EEAe (ppm)			
	0 (control)	100	250	450
No. mated	8	7	7	8
No. pregnant	5	7	7	7
No. pregnant with live foetuses at term	4	5	7	5
No. sick (values excluded)	0	0	0	1
No. abortions (values excluded)	0	2	0	0
Mean no. implantations	7.4 ±1.1 (5)	7.0 ±1.6 (5)	6.7 ±2.6 (7)	7.2 ±1.9 (6)
Pre-implantation loss:				
Mean %	0.0 ±0.0 (5)	15.0** ±9.9 (5)	19.4 ±25.5 (7)	27.1* ±25.4 (6)
% litters affected	0.0	80.0+	57.1	83.3+
Mean no. live foetuses/litter	6.0 ±3.5 (5)	6.2 ±1.9 (5)	6.0 ±2.4 (7)	4.3 ±3.0 (6)

- * Statistically significantly different from the control group mean at the 5% level (t-test, one-sided)
 ** Statistically significantly different from the control group mean at the 1% level (t-test, one-sided)
 + Statistically significantly different from the control group mean at the 5% level (Fisher's exact test, one-sided)

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 9 - continued

LITTER DATA

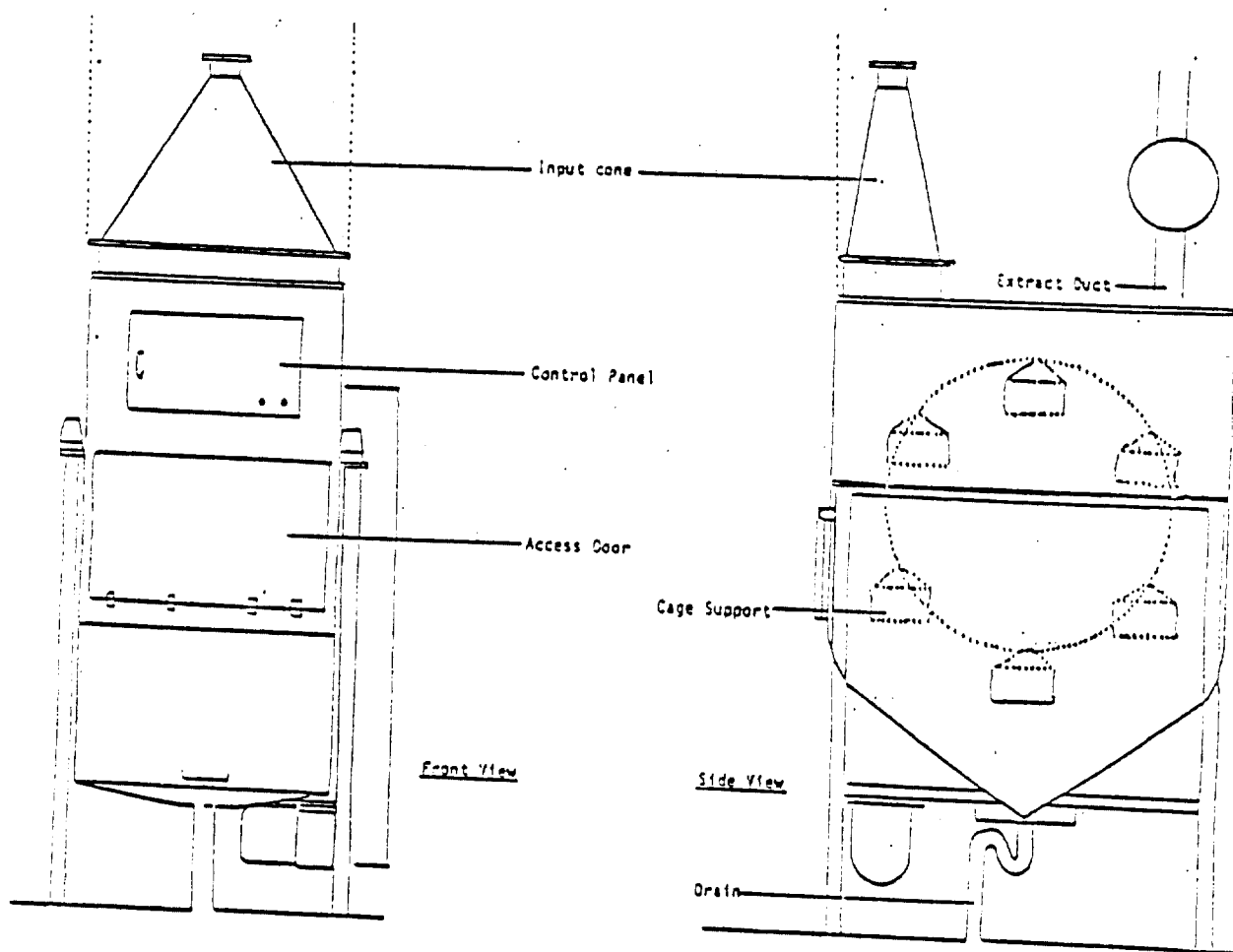
	Exposure concentration of EEAe (ppm)			
	0 (control)	100	250	450
Post implantation loss:				
Mean %	20.0 ±44.7 (5)	11.7 ±16.2 (5)	11.3 ±13.3 (7)	37.5 ±39.4 (6)
% litter affected	20.0	40.0	57.1	66.6
Intra-uterine deaths:				
Mean early	0.0 ±0.0 (5)	0.6 ±0.9 (5)	0.4 ±0.5 (7)	2.3* ±2.7 (6)
Mean late	1.4 ±3.1 (5)	0.2 ±0.4 (5)	0.3 ±0.5 (7)	0.5 ±0.8 (6)
Mean foetal wt (g) /litter	5.2 ^a ±0.2 ^a (4)	4.3** ±0.4 (5)	4.2** ±0.3 (7)	3.4** ±0.6 ^a (5)
Mean gravid uterine wt (g)	121.2 ±46.1 (5)	113.7 ±21.5 (5)	116.9 ±42.0 (7)	70.7* ±30.5 (6)

* Statistically significantly different from the control group mean at the 5% level (t-test, one-sided)
 ** Statistically significantly different from the control group mean at the 1% level (t-test, one-sided)
 a - one rabbit had no live foetuses.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

FIGURE 1

EXPOSURE CHAMBER



ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 1

ANALYTICAL DETAILS OF THE TEST MATERIAL USED IN THE STUDY

Description	Clear, free from suspended matter
Colour (Hazen Units)	<5
Water content (% w/w)	0.02
Ester content (% w/w)	99
Acidity (% w/w as acetic acid)	0.010
Specific gravity (20/20°C)	0.975
Residue on evaporation (% w/w)	<0.001
Distillation range °C	
Initial boiling point	155.2
Dry point	159.3

Confirmation of identity was by comparison with a reference infra-red spectrum of EEAc.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 2

CHEMICAL COMPOSITION OF LABORATORY ANIMAL DIETS (the vitamin and trace mineral composition of pelleted diets refers to the amounts of each nutrient added to the diet and ignores the natural sources).

Diet CRB (Rabbit Breeder)

Calculated Analysis

Crude Oil	1.9%
Crude Protein	16.1%
Crude Fibre	14.0%
Calcium (as Ca)	1.1%
Phosphorus (as P)	0.6%
Salt (as NaCl)	0.7%
Metaboliseable Energy	1934 Kcal/kg
Carbohydrate	50.27%

Trace Elements Added

Manganese	125ppm
Copper	7ppm
Cobalt	0.4ppm
Iron	30ppm
Iodine	1.3ppm
Magnesium	102ppm

Vitamins Added (per kg)

Vitamin A	8000IU
D ₃	1000IU
B ₂	8mg
Nicotinic Acid	50mg
Pantothenic Acid	12mg

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 2 - continued

Vitamins Added (per kg)

Vitamin B ₁₂	12µg
E	60I.U.
K	10mg
Folic Acid	10mg
Choline chloride	200mg
Vitamin B ₁	4mg
Vitamin B ₆	6mg

Amino Acids (as percentage of feed)

Threonine	0.6
Glycine	0.8
Valine	0.8
Cystine	0.2
Methionine	0.2
Isoleucine	0.7
Leucine	1.2
Tyrosine	0.6
Phenylalanine	0.7
Lysine	0.9
Histidine	0.4
Arginine	1.1
Tryptophan	0.1

An analysis of each batch of diet for major constituents and contaminants was supplied by CRB. This was checked for acceptability, based on the best available information at the time, before the batch was used on the study. The known contaminants found in the diet were considered not to be present in sufficient concentration to have had an influence on the outcome of the study.

Water

Analyses of tap water were carried out periodically and checked for acceptability. The known contaminants found in the water were considered not to be present in sufficient concentration to have had an influence on the outcome of the study.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 3

TEMPERATURE AND RELATIVE HUMIDITY IN THE EXPOSURE CHAMBERS

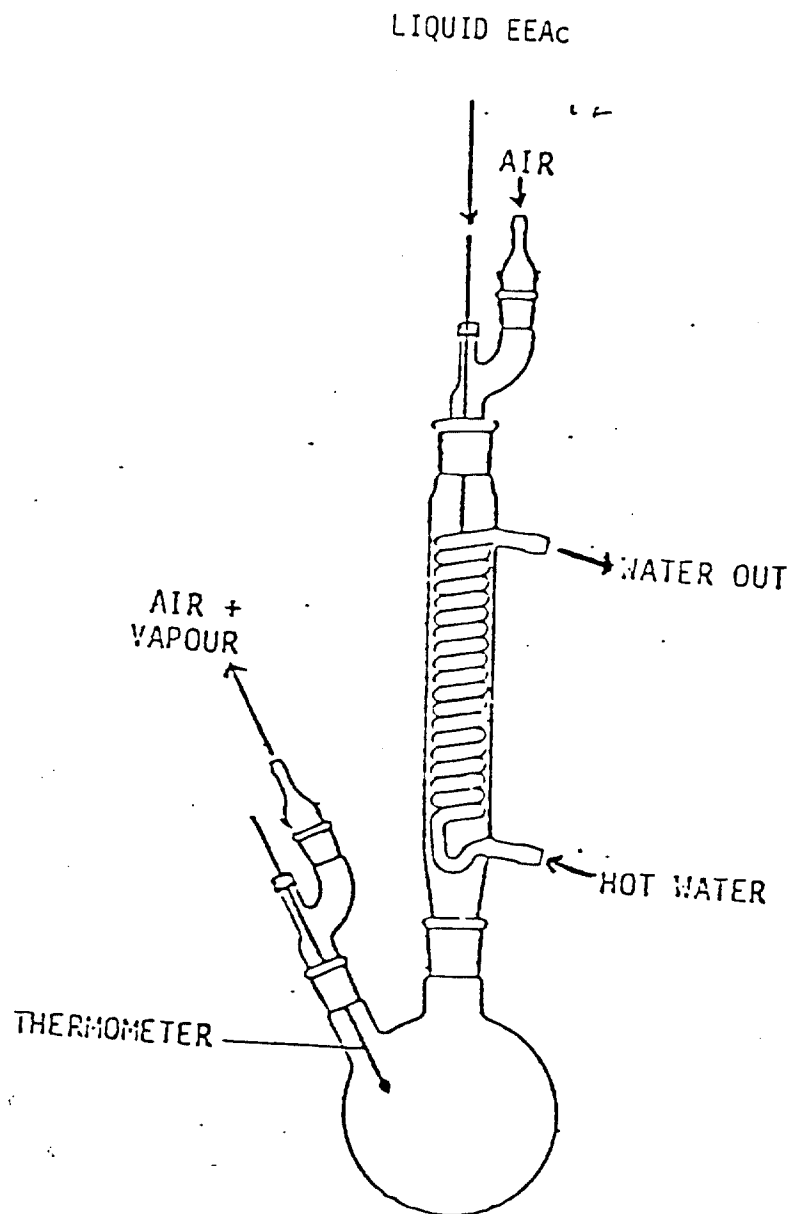
Exposure concentration of EEAe (ppm)

	0 (control)		100		250		450	
	Temp °C	RH%	Temp °C	RH%	Temp °C	RH%	Temp °C	RH%
Range	22.25-23.25	48-73	22.25-23.25	51-69	22.25-23.5	52-69	22.5-23.5	48-66
Mean	22.7	59.0	22.7	59.3	22.9	60.2	23.2	57.6
SD	0.3	6.1	0.3	4.8	0.3	4.7	0.3	5.3
n	16	16	16	16	16	16	16	16

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 4

METHOD OF ATMOSPHERE GENERATION FOR EEAc



The generation system for each exposure level consisted of a reservoir of EEAc, a peristaltic pump (GILSON) a glass condenser, and a round-bottom flask.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
PROBE INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 4 - continued

METHOD OF ATMOSPHERE GENERATION FOR EEAc

The EEAc was metered from the reservoir onto the condenser coil by means of the peristaltic pump fitted with solvent resistant tubing (ISOVERSINIC, GILSON). Hot water 35-40°C from a thermocirculator (CHURCHILL) was circulated through the condenser coil to aid in the volatilisation of the EEAc.

A carrier air flow of 2-6l/min was passed down the condenser jacket and through the flask. The carrier air plus the EEAc vapour was then passed into the input air of the chamber.

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY 8 REPORT (CONFIDENTIAL)
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Sponsor: Chemical Manufacturers*
Association Washington
DC 20037

CTL Ref: Y01321/002

Study No: RB0222

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REPORT NO: CTL/P/840

ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE (EEAc): INHALATION TERATOGENICITY
STUDY IN RABBITS

by

D J Tinston
J E Doe
M Killick
M Thomas

* This work was carried out for the Glycol Ethers Program Panel of the
C M A under Contract No. GE -11.0-Ter-Ih1-ICI

Approved for Issue: *[Signature]*

Date of Issue: 25 AUG 1983

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

QUALITY ASSURANCE STATEMENT

The conduct of this study has been inspected/audited by the CTL Quality Assurance Unit as follows:

Date	Inspection/Audit	Date of QA Report
3 Jun 82	Inspection	3 Jun 82
29 Jun 82	Protocol Audit	29 Jun 82
5 Jul 82	Inspection	5 Jul 82
15 Jul 82	Inspection	15 Jul 82
20 Jul 82	Inspection	30 Jul 82
22 Jul 82	Inspection	2 Aug 82
23 Jul 82	Inspections	23 Jul 82
10 Aug 82	Inspection	10 Aug 82
26 May 83	Draft Report Audit	27 May 83
19 Aug 83	Final Report Audit	19 Aug 83

Inspection has been carried out and the report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice.

J R Pateman (Unit Head, CTL Quality
Assurance Unit)

J R Pateman 19.8.83....

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

We, the undersigned, declare that this report constitutes a true record
of the actions taken and the results obtained in the above study.

J E Doe	Study Director	<i>[Signature]</i>	12 Aug 83
D J Tinston	Study Investigator	<i>David J. Tinston</i>	19 Aug 83
M Killick	Teratologist	<i>M Killick</i>	19 Aug 83
M Thomas	Statistician		
D M Samuels	Study Pathologist	<i>D M Samuels</i>	22 Aug 83
I S Chart	Haematologist	<i>I S Chart</i>	23 Aug 83
G A Wickramaratne	Senior Toxicologist	<i>[Signature]</i>	23 Aug 83

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

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ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

SUMMARY

Groups of pregnant Dutch rabbits were exposed to 0 (Controls), 25, 100 or 400ppm of EEAc for 6 hours a day on days 6-18 (inclusive) of gestation. On day 29 of gestation, the rabbits were killed and their ovaries and uterine contents were examined.

Exposure to 400ppm EEAc caused reduced maternal weight gain and food consumption and also reduced the blood haemoglobin level but there were no clinical abnormalities which could be attributed to treatment. There were no effects upon the dams at 100ppm or 25ppm EEAc.

There was an increase in the number of rabbits with total resorptions at 400ppm EEAc and a reduction in the weights of the surviving foetuses. There was also a reduction in foetal weight at 100ppm EEAc, which may be associated in part with increased litter size at this concentration. Exposure to 25ppm EEAc had no effect on foetal numbers or foetal weight.

In the foetuses from the rabbits exposed to 400ppm EEAc there was retarded ossification indicative of foetotoxicity. There was slight foetotoxicity at 100ppm EEAc in the form of retarded ossification, but there was no evidence of foetotoxicity at 25ppm EEAc.

At 400ppm EEAc there was some evidence of teratogenicity as shown by major malformations of the vertebral column. There was no evidence of teratogenicity at either 100ppm EEAc or 25ppm EEAc.

1. INTRODUCTION

Ethylene glycol monoethyl ether acetate (EEAc, 2-ethoxyethanol acetate) is a solvent for nitrocellulose, low viscosity cellulose and resins. EEAChas a boiling point of 156°C at 760mm Hg, and a vapour pressure of 2mm Hg at 20°C. Inhalation could, therefore, be a route of exposure for users of this material.

Truhaut et al (1979) reported that rats and rabbits exhibited transient haematuria when exposed to the saturated vapour of EEACh for four hours. However, haematuria, ketonuria, haematological changes and bodyweight effects were not seen in rats and rabbits exposed to 200ppm of EEACh, four hours/day, five days/week for ten months. (Truhaut et al, 1979).

Nagano et al (1979) gavaged mice with EEACh five days/week for five weeks; 1000mg/kg/day caused testicular atrophy and leukopenia was seen at 2000mg/kg/day. Nelson et al (1982) found that EEACh was teratogenic in rats.

The study described in this report was designed to assess the effects of inhalation of EEACh on pregnant Dutch rabbits in order to define no-effect exposure levels for embryo/foetotoxicity and teratogenicity.

A preliminary study conducted at Central Toxicology Laboratory (Tinston, 1983) demonstrated maternal toxicity (reduced bodyweight gain and food consumption) and foetotoxicity (reduced foetal weights) at 100, 250 and 450ppm of EEACh. Exposure levels of 25, 100 and 400ppm of EEACh were chosen for the present study based on the results of the preliminary study.

The exposures to EEACh started on 13 July 1982 and the final post mortem examinations were conducted on 20 August 1982.

All original data relating to this study are maintained in the Archives of Central Toxicology Laboratory. Copies of this report are held by the Reports Centre, Central Toxicology Laboratory.

The study was sponsored by the Glycol Ethers Program Panel of the Chemical Manufacturers Association, Washington, D.C.

2. EXPERIMENTAL PROCEDURES

2.1 Test Material

EEAc was assigned a Central Toxicology Laboratory reference number of Y01321/002. The test material was 99% pure and was supplied by, Imperial Chemical Industries PLC, Petrochemicals and Plastics Division, Wilton, Middlesbrough, UK. Analytical details are shown in Appendix 1.

2.2 Animals and Husbandry

Virgin, female Dutch rabbits (5-7 months old, 1.7 - 2.8kg in weight) were used. They were supplied by Ranch Rabbits, Crawley Down, Sussex, UK, and delivered to the experimental unit where they were housed individually in cages (47 x 45 x 58cm) in mobile rabbit racks. Each rabbit was identified by a numbered metal ear tag. They were acclimatised to the exposure chambers (see 2.5), where they were individually housed in wire mesh cages (45 x 21 x 20cm) for no more than 6 hours/day for at least 6 days prior to mating. During the acclimatisation period the clinical condition of each rabbit was monitored. Twelve bucks of the same strain and of proven fertility, supplied by the same breeder, were housed within the same experimental unit. Food (CR8 pellets) supplied by Labsure Animal Diets, Poole, Dorset, UK, and tap water (Appendix 2) were available for all animals ad libitum except when housed in exposure chambers. The temperature and relative humidity within the animal room were maintained at nominal levels of 21°C and 50% to 70% respectively. Readings were taken throughout the study by a wet and dry bulb thermometer (Appendix 3). A 12 hour light (starting at 06.00 hours) and 12 hour dark cycle was also maintained.

2.3 Study Design

Table 1 shows the animal numbers assigned to each group.

TABLE 1

Group	Exposure concentration of EEAc(ppm)	Experimental animal numbers
1	0 (Air only)	1 - 24
2	25	25 - 47
3	100	49 - 71
4	400	73 - 96

2.4 Mating

Each doe was placed with a buck and coitus observed. A vaginal smear was taken from the first doe each buck mated with and the smear was examined for the presence of motile sperm. Within 5 hours of mating each doe received an intravenous injection of 25 IU of chorionic gonadotrophin ('PREGNYL' Organon Laboratories Limited, Morden, Surrey, UK) to promote ovulation. Matings took place over a two week period on four consecutive days within each week and ten or twelve does were mated on each day. The sequence of allocation of does to groups is shown in Appendix 4. The day of mating was termed Day 0 of pregnancy. Following mating, the does were placed in exposure chambers (see 2.5) for 6 hours/day until day 18 of gestation, but were not exposed to the test material until day 6 of gestation.

2.5 Exposure Regime

On days 6-18 (inclusive) of gestation the rabbits in groups 2, 3 and 4 were exposed to the appropriate concentration of EEAc for 6 hours/day in exposure chambers (Doe and Tinston, 1981). The rabbits in group 1 were also placed in chambers of the same design but they were exposed to air only.

The chambers (Figure 1) had an internal volume of approximately 3.4 m^3 . They were constructed of stainless steel and access was gained to each

chamber through a door fitted with a safety glass window. Air entered at the front of each chamber and was extracted at the back. Within each chamber were six cage levels and excreta collection trays which rotated concurrently (0.5 times per minute) with the direction of flow of the input air. The air flow rate was set to 600 l/min approximately using a flowmeter (ROTAMETER). Data describing the distribution of EEAc within these chambers are shown in Appendix 5.

The chamber air supply was conditioned nominally to 21°C and 50% relative humidity; the temperature and relative humidity in each chamber were recorded daily (Appendix 3).

2.6 Atmosphere Generation and Analysis

Dynamic atmospheres of EEAc were generated by metering appropriate amounts of EEAc vapour into the input air of each chamber. The method of generation of EEAc vapour is described in Appendix 6.

The concentration of EEAc in each chamber was analysed between nine and nineteen times per exposure day. Each of the chambers had a sampling point in the back wall connected by 4mm i.d. PTFE tubing to an infra-red analyser (WILKS MIRAN). Two analysers were used, one for the 25ppm level and one for the 100 and 400ppm levels. Calibration procedures and analyser conditions are detailed in Appendix 7.

Daily nominal concentrations of EEAc in each chamber were also calculated based on the rate of use of EEAc.

2.7 Maternal Bodyweights, Food Consumption and Clinical Observations

The clinical condition of all animals was recorded daily and they were observed during each exposure period for any abnormalities.

The bodyweight of each rabbit was recorded on Days 0, 5-19, 24 and 28 of pregnancy.

Food consumption was measured by giving each rabbit a weighed amount of food on Days 0, 5, 10, 14, 19 and 24 of gestation. The amount consumed was calculated from the amount left on Days 5, 10, 14, 19, 24 and 28 of gestation. Food wasted was not measured, but was minimal.

2.8 Terminal Investigations

All rabbits apart from one which died on day 26 were killed on day 29 of pregnancy by inhalation of halothane B P (FLUOTHANE Imperial Chemical Industries PLC) and were subjected to a post mortem examination.

Blood samples were taken by cardiac puncture and placed in pots containing EDTA. The following assays were carried out using a Coulter 'Model S': haemoglobin, haematocrit, total white cell count, red cell count, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration. Femoral bone marrow and blood smears were also prepared but were not subsequently examined.

Spleens were weighed and stored with any abnormal tissues in formal saline but the tissues were not subsequently examined microscopically.

The uterus was dissected out and the gravid uterus was weighed. The number of corpora lutea in each ovary was counted. The uterus was opened by an incision on the abendometrial wall and the numbers of implantations, early intra-uterine deaths and late intra-uterine deaths were counted. Intra-uterine deaths were identified as being late when foetal tissues were distinguishable. Each live foetus was removed from the uterus by severing the umbilical cord. The foetuses were assigned letters of the alphabet to identify their position in utero starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. Each foetus was then weighed, killed with an intra-cardiac injection of pentobarbitone sodium (EUTHATAL), examined externally for gross abnormalities including cleft palate and identified within the litter by limb tagging.

2.9 Assessment of Foetuses

2.9.1 Visceral Examinations: The skin was removed from each foetus and the eyes were examined for any abnormalities. The major organs of

the abdomen and thorax were examined for abnormalities and the sex of each foetus was determined. Any abnormalities and the position of the major blood vessels in the thorax and their emergence from the heart were noted. A section across the heart was made to check for internal defects. All the foetuses were eviscerated and placed in 70% methanol.

After approximately 24 hours, an incision was made along the fronto-parietal suture line of each foetus in order to examine the brain for gross defects.

2.9.2 Skeletal Examinations: All foetuses were cleared, stained with Alizarin red S (Staples and Schnell, 1964) and examined to assess morphological development and the degree of ossification. The individual bones of the manus and pes were assessed and the result converted to a five point scale as detailed in Appendix 3.

2.9.3 Classification of Defects: Defects were classified as major (rare or possibly lethal or both) or minor (deviations from normal that are common at external, visceral or skeletal examination). Variations in the degree of ossification of the foetuses were also recorded and classified as minor defects or variants depending on the frequency of occurrence in historical controls. Extra thoracic ribs were classified as variants.

2.10 Statistical Analyses

2.10.1 Maternal Bodyweight Gain, Food Consumption, Haematology, Spleen Weight and Litter Data: Data from non-pregnant animals and animals with abortions were excluded from the analysis.

Differences between the experimental groups were investigated using the analysis of variance. Individual treatment group means were compared with the control group using Student's 't' test, based on the error mean square from the analysis of variance. All 't' tests were one-sided, except for: bodyweight gain, food consumption, haematology parameters and the proportion of foetuses which were male.

Maternal spleen weight was assessed by analysis of variance and covariance on final bodyweight. Spleen weight relative to bodyweight was also assessed by analysis of variance.

Data recorded as proportions for each dam (eg proportion of male foetuses) were subjected to the single arcsine transformation (Fisher, 1954) to stabilise the variance before analysis. The analysis of variance was weighted by the denominator of the proportion. The analysis of variance for mean foetal weight was weighted by the number of viable foetuses per litter.

Data recorded as proportions of litters or foetuses responding in each group were investigated using Fisher's exact test. Individual treatment group proportions were compared with the control group proportions. All comparisons were one-sided.

Percentage pre- and post-implantation losses were calculated from the following formulae:

% pre-implantation loss =

$$\frac{(\text{number of } \underline{\text{corpora lutea}} - \text{number of implantations}) \times 100}{\text{number of } \underline{\text{corpora lutea}}}$$

% post-implantation loss =

$$\frac{(\text{number of implantations} - \text{number of live implantations}) \times 100}{\text{number of implantations}}$$

When, the number of corpora lutea for a particular animal, was recorded as less than the number of implantations, the number of corpora lutea was assumed equal to the number of implantations, and the pre-implantation loss was taken as zero. The transformed pre- and post-implantation losses were calculated on a litter basis, but untransformed means were calculated on a group basis. This is consistent with the weighting in the analysis of variance of transformed proportions.

2.10.2 Skeletal Data, Visceral and External Foetal Defects: The incidence of each external/visceral and skeletal defect was assessed using Fisher's exact test. The proportion of foetuses affected in each treated group was compared with the proportion of foetuses affected in the control group. All comparisons were one-sided. The comparisons were

repeated, using the proportion of litters showing each defect. These latter comparisons are not tabulated since they lead to the same conclusions as those based on the proportion of fetuses affected.

Several of the more common defects were selected for further analysis. The proportion of fetuses affected in each litter was subjected to the single arcsine transformation (Fisher, 1954). The analysis of variance was used to assess inter-group differences in the transformed proportion. The analysis was weighted by the number of fetuses examined. The results of this analysis are not tabulated as they lead to the same inferences as the Fisher's exact tests.

The proportions of fetuses in each litter with any external/visceral defect, any major external/visceral defect, any skeletal variant, any skeletal defect and any major skeletal defect were transformed using the single arcsine transformation. Transformed proportions were assessed using the analysis of variance, which was weighted by the number of fetuses examined. Following the analysis of variance, mean transformed proportions in each treated group were compared with mean transformed proportions in the control group using Student's 't' test. The 't' statistic was based on the error mean square from the analysis of variance, and all comparisons were one-sided. In addition, untransformed proportions were compared using Fisher's exact test.

The average manus and pes score for each litter was investigated using the analysis of variance. The analysis was weighted by the number of viable fetuses per litter. Following the analysis of variance, individual treatment group means were compared with the control group mean using Student's 't' test : based on the error mean square from the analysis of variance. All comparisons were one-sided.

3. RESULTS

3.1 Atmosphere Analysis (Tables 2 and 3)

The daily mean analysed concentrations were all within $\pm 5\%$ of target except for 18 July at 25ppm (+ 3%), 18 July at 100ppm (+6 %) and 7, 8 August at 400ppm (+ 7 and + 5%).

At 400 and 100ppm there was a good correlation between the concentrations as determined by chemical analysis and those calculated from weight loss of the test material. At 25ppm the calculated concentrations were 40% higher than the analysed concentrations but it was found that at this exposure level approximately 30% of the test material was accounted for in priming the generation system tubing.

Therefore, the analysed concentrations and the nominal concentrations indicate that the rabbits were exposed to concentrations of EEAc which were close to the target levels.

3.2 Maternal Bodyweight Gain, Food Consumption and Clinical Observations

3.2.1 Maternal Bodyweight Gain (Table 4): There was a dose-related adverse effect on bodyweight in all three groups exposed to EEAc compared to controls during the thirteen days of exposure, but this was statistically significant only in the 400ppm group. The reduction was mainly confined to the first few days of exposure with subsequent recovery towards control values. In the 400ppm group, bodyweight gain during the post-exposure period was statistically significantly higher than the controls.

3.2.2 Maternal Food Consumption (Table 5): There was a dose-related decrease in the food consumed by all three groups exposed to EEAc during the exposure period compared with controls, but this was statistically significant only in the 400ppm group. During the post-exposure period the food consumed by the 100ppm group remained slightly lower than the controls whilst that of the 400ppm group was statistically significantly higher than the controls.

3.2.3 Clinical Observations: There were no changes in clinical condition or behaviour which could be related to exposure to EEAc, at any of the concentrations used in the study.

One animal (number 10, Control group) was found dead on day 26 of gestation and evidence of abortion was found at autopsy. This animal had previously lost weight and a yellow-coloured vaginal discharge was observed on the day before it was found dead. One animal (number 55,

100ppm group) aborted on day 24 of gestation but otherwise appeared healthy apart from some hair loss. Of the four animals with total resorptions, vaginal bleeding was observed in number 95 (400ppm group), weight loss and lethargy was observed in number 82 (400ppm group), and fatty tissue was found on the excreta collection tray of number 38 (25ppm group). There were no clinical abnormalities in the other rabbit (number 86, 400ppm group) with total resorption.

3.3 Terminal Investigation

3.3.1 Macroscopic Observations In the Does (Table 6): Neither the incidence nor the type of maternal macroscopic observations could be related to exposure to EEAc.

3.3.2 Spleen Weights (Table 7): There was no evidence for any effects on spleen weights as a result of exposure to EEAc.

3.3.3 Haematology (Table 8): In the 400ppm group there was a statistically significant reduction in haemoglobin concentration and a slight but not statistically significant reduction in the associated red blood cell parameters (haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration) compared with control values. There were also minor reductions in the same red blood cell parameters in the 100ppm group but none of these was statistically significant.

There was a dose-related decrease in white blood cell count in all three groups exposed to EEAc although these were not statistically significantly different from controls.

3.3.4 Litter Data (Table 9): There was an increased incidence of intra-uterine deaths in the 400ppm group which was associated with a statistically significantly higher percentage post-implantation loss and a statistically significantly lower mean number of live fetuses per litter compared with controls. In order to determine whether the increased incidence of uterine deaths was due solely to the three animals in the 400ppm group with total resorptions, the data were reanalysed omitting these animals. When the data from these three animals with total resorptions were omitted from the 400ppm group there were no

statistically significant differences in percentage post-implantation loss and mean number of live fetuses per litter compared with controls.

Mean live foetal weight was statistically significantly reduced in the 100 and 400ppm groups, but mean total litter weight was statistically significantly lower than controls only in the 400ppm group.

There were no statistically significant differences from control values in any of the litter data parameters in the 25ppm group and the omission of one animal with total resorptions had little effect on the data.

3.3.5 Foetal External/Visceral and Skeletal Defects (Tables 10-13)
Major Defects: The incidence of fetuses with major defects was control group: 1, 25ppm group: 1, 100ppm group: 2 and 400ppm group: 8. In the 400ppm group the fetuses with major defects were from four different litters: two of the 8 fetuses had major external or visceral defects (brain ventricles moderately dilatated, both forelimbs malrotated), 5 had major skeletal defects associated with the vertebral column with misaligned vertebral arches and an additional hemi-vertebra. One fetus had a major visceral defect (agenesis of the right kidney) and a major skeletal defect (of a similar nature to the others).

There were no major skeletal defects in the 25 or 100ppm groups. One fetus from the 25ppm group had agenesis of the left kidney, one fetus in the 100ppm group had an ovary attached to the intestine and another fetus in the 100ppm group had its right forelimb malrotated. In the control group, one fetus had a major vertebral defect.

Minor Defects: In the 400ppm group there was a statistically significant increase in the proportion of fetuses with minor external/visceral defects in comparison with the control group. Within this 400ppm group there was a statistically significant increase in the proportion of fetuses with pelvic dilatation, opaque/empty gall bladders, pale and reduced spleens.

In the 100ppm group the proportion of fetuses with pale spleens was also statistically significantly increased in comparison with the control group.

There were no statistically significant increases in the proportion of fetuses with any specific external or visceral defect in the 25ppm group when compared with the controls.

The proportion of fetuses with any external or visceral defect was statistically significant only in the 400ppm group in comparison with the controls.

In addition there was a statistically significant increase in the proportion of fetuses with minor skeletal defects in the 400ppm group in comparison with the control group. In this 400ppm group specific minor skeletal defects indicative of retarded ossification were statistically significantly increased in comparison with the controls. In addition, the incidence of 27 presacral vertebrae was statistically significantly increased compared with controls, and this was related to the high incidence of extra ribs.

In the 100ppm group the proportion of fetuses with minor skeletal defects was slightly increased but the difference from the control group did not attain statistical significance. Only two specific defects, partial ossification of the 1st cervical centrum and the 2nd sternbra, were statistically significantly higher in the 100ppm group than in the control group.

The incidence of one minor defect, an extra centre of ossification situated above the 1st sternbra, was statistically significantly higher in the 25ppm group than in the control group. This finding is considered to be of no biological or toxicological significance since a lower incidence was found in the 100ppm group and none were found in the 400ppm group. There was, therefore, no evidence of a dose-response relationship for this defect.

In the assessment of the manus and pes, statistically significant differences from the control group were observed for the manus and pes in the 100ppm group and for the pes in the 400ppm group. The higher scores indicate poorer ossification of the manus or pes although intergroup differences were small.

Variants: The proportion of fetuses with skeletal variants was statistically significantly higher in both the 100 and 400ppm groups than

in the control group . In these groups the incidence of 13 bilateral ribs was statistically significantly increased and markedly so in the 400ppm group. The incidence of partial ossification of the 5th sternebra was also statistically significantly increased in the 400ppm group.

In the 25ppm group there were no statistically significant differences from the control group.

4. DISCUSSION

Exposure to 400ppm of EEAc caused some mild toxicity in the pregnant rabbits since there was an adverse effect on weight gain during the exposure period, their food consumption was reduced and there was a marginal reduction in blood haemoglobin concentration. There was no evidence of maternal toxicity in the pregnant rabbits exposed to either 25 or 100ppm EEAc apart from non-statistically significant bodyweight reductions during the first three days of exposure.

The occurrence of total litter resorption in three dams in the 400ppm EEAc group cannot be conclusively associated with exposure to EEAc as there was no concomitant increase in post-implantation loss in those females with live fetuses in utero. However, the reduction in mean litter weight as a result of reduced foetal weight is evidence of foetotoxicity at 400ppm. There was also a reduction in mean foetal weight but not litter weight in the group exposed to 100ppm EEAc. While the latter may be attributable, in part, to the higher proportion of litters containing a large number of fetuses (ten or more) and therefore causing intra-litter competition, it may be evidence of a slight foetotoxic effect at 100ppm EEAc. There was no evidence from the litter data of a foetotoxic effect at 25ppm EEAc.

Examination of the fetuses from the rabbits exposed to 400ppm EEAc confirmed foetotoxicity as indicated by reduced foetal weight. There was reduced skeletal ossification in both the vertebrae and the sternebrae, increased numbers of thoracic ribs, and an increased incidence of slight renal pelvic dilatation all of which are indicative of foetotoxicity. The increased incidences of opaque gall bladders and smaller spleens

are difficult to interpret, as the biological significance of this is not known.

There was also some evidence of foetotoxicity from the skeletal examination of the foetuses from the rabbits exposed to 100ppm EEAc. There was an increased incidence of extra thoracic ribs and some retarded ossification in the vertebral column and the sternbrae. These increases in minor defects and variants only occurred in statistically significant numbers where there was a greater incidence at 400ppm EEAc and they therefore appeared to form part of a dose-response relationship. None of these effects were seen in the foetuses of rabbits exposed to 25ppm EEAc and this concentration appears not to be foetotoxic. The incidence of an extra centre of ossification above the first sternbra was low and although it was statistically significant, did not form part of a dose-response curve and is therefore not considered to be evidence of foetotoxicity.

At 400ppm EEAc there were 5 foetuses with major skeletal malformations involving the vertebral column; one foetus had a similar major skeletal malformation and agenesis of one kidney; one foetus had moderate dilatation of the brain ventricles; and one had malrotation of the forelimbs. The incidence of six major skeletal malformations compared with one in the controls provide evidence of possible teratogenicity at 400ppm EEAc. The incidences of major external and visceral abnormalities do not provide evidence for teratogenicity at 400ppm EEAc. There was no evidence of teratogenicity at either 100ppm or 25ppm EEAc.

Although there were no cardiac abnormalities in this study, the results are broadly similar to those reported by Nelson et al, 1982. Rats were exposed to 600ppm, 390ppm or 130ppm of EEAc. There was complete resorption of all implants at 600ppm EEAc. At 390ppm EEAc there was foetotoxicity in the form of delayed ossification and cardiac abnormalities as evidence of teratogenicity. At 130ppm EEAc there was only one major visceral abnormality (a cardiac abnormality), and evidence of foetotoxicity ie an increased incidence of skeletal defects, mainly delayed ossification. The two studies together indicate that 390-400ppm EEAc is teratogenic, 100-130ppm EEAc is foetotoxic and the study reported here indicates that 25ppm EEAc is a no-effect level.

5. CONCLUSIONS

Exposure of pregnant rabbits to 400ppm EEAc caused maternal toxicity, foetotoxicity and possible teratogenicity. Exposure to 100ppm EEAc caused slight foetotoxicity, but exposure to 25ppm EEAc did not. There was no evidence of teratogenicity at either 100ppm EEAc or 25ppm EEAc.

DJT/SHR (237)

16.08.33

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ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 2
DAILY ANALYSED CHAMBER CONCENTRATIONS

Date		Target Concentration 25ppm	Target Concentration 100ppm	Target Concentration 400ppm
18.7.82	Mean SD n	27.0 4.5 13	106 9 12	412 51 13
19.7.82	Mean SD n	23.8 2.8 17	97 13 12	404 45 13
20.7.82	Mean SD n	25.2 3.3 16	103 13 11	408 49 12
21.7.82	Mean SD n	24.6 4.7 16	97 13 9	411 37 10
22.7.82	Mean SD n	23.9 1.0 16	103 12 10	420 19 11
23.7.82	Mean SD n	24.6 2.0 18	97 9 12	411 25 13
24.7.82	Mean SD n	25.1 0.9 17	100 15 12	415 27 13
25.7.82	Mean SD n	24.5 2.3 18	100 9 12	417 28 13
25.7.82	Mean SD n	24.2 1.3 18	97 13 12	415 26 13
27.7.82	Mean SD n	25.1 1.2 17	97 9 12	412 40 13
29.7.82	Mean SD n	25.0 1.7 18	98 11 12	415 27 13

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 2 - continued

DAILY ANALYSED CHAMBER CONCENTRATIONS

Date		Target Concentration 25ppm	Target Concentration 100ppm	Target Concentration 400ppm
29.7.82	Mean SD n	25.1 1.4 17	99 8 13	410 22 13
30.7.82	Mean SD n	25.0 1.3 17	99 13 12	409 36 13
31.7.82	Mean SD n	25.2 1.9 17	100 10 12	404 42 13
01.8.82	Mean SD n	24.1 3.1 17	97 8 12	414 30 13
02.8.82	Mean SD n	25.3 2.5 17	97 17 13	411 32 13
03.8.82	Mean SD n	25.1 2.8 17	98 15 12	413 36 13
04.8.82	Mean SD n	24.5 2.9 16	99 14 12	400 52 13
05.8.82	Mean SD n	24.5 4.3 16	98 13 13	417 45 13
06.8.82	Mean SD n	25.3 1.1 19	100 13 13	411 56 13
07.8.82	Mean SD n	25.7 1.3 17	101 11 13	426 34 14

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 2 - continued

DAILY ANALYSED CHAMBER CONCENTRATIONS

Date		Target Concentration 25ppm	Target Concentration 100ppm	Target Concentration 400ppm
08.8.82	Mean SD n	25.3 1.3 18	102 7 13	421 19 14
09.8.82	Mean SD n	24.9 1.9 18	99 9 12	393 46 13
Overall	Mean SD n	24.9 0.7 23	99 2 23	412 7 23

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 3
DAILY NOMINAL CHAMBER CONCENTRATIONS OF EEAc

Date	Target Concentration of EEAc(ppm)		
	25	100	400
18.7.82	40.8	100	365
19.7.82	37.5	100	362
20.7.82	33.5	97	359
21.7.82	33.8	99	388
22.7.82	29.9	98	361
23.7.82	32.8	111	369
24.7.82	33.2	96	362
25.7.82	31.5	105	375
26.7.82	33.5	92	377
27.7.82	33.6	97	371
28.7.82	38.7	95	370
29.7.82	32.7	96	361
30.7.82	33.1	95	365
31.7.82	36.6	98	355
01.8.82	37.0	94	362
02.8.82	40.6	95	411
03.8.82	34.8	95	368
04.8.82	44.2	101	379
05.8.82	35.0	99	378
06.8.82	32.5	98	419
07.8.82	32.6	98	368
08.8.82	31.5	95	367
09.8.82	33.4	93	377
Overall mean	34.9	98	373
standard deviation	3.5	4	16
Overall mean nominal/ overall mean analysed	1.4	1.0	0.9

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 4

INTERGROUP COMPARISON OF MATERNAL BODYWEIGHT GAIN (g)

Period	Exposure Concentration of EEAc (ppm)				Approximate ¹ 95% confidence interval
	0(control)	25	100	400	
Initial bodyweight					
Mean	2132	2124	2153	2197	
SD	304	174	208	235	
Before exposure:					
Day 0-Day 5					
Mean	32.4	50.2	46.2	58.1	±26.0
SD	43.1	42.0	59.8	61.7	
During Exposure:					
Day 5-Day 8					
Mean	-5.8	-10.9	-33.5	-61.4**	±22.4
SD	25.6	26.4	59.7	55.4	
Day 8-Day 11					
Mean	27.9	19.3	29.4	-0.7	±23.0
SD	35.6	29.3	54.7	58.3	
Day 11-Day 18					
Mean	72.7	65.0	66.4	60.6	±41.4
SD	76.1	64.6	86.0	102.3	
Day 5-Day 18					
Mean	94.8	73.9	62.3	-1.5*	±54.3
SD	109.8	91.5	117.0	123.4	
After exposure:					
Day 18-Day 28					
Mean	147.6	160.9	138.9	211.4*	±44.0
SD	87.4	82.1	116.8	68.4	
Number females	16	15	17	19	

* Statistically significantly different from the control group mean at the 5% level (t-test, two-sided).

** Statistically significantly different from the control group mean at the 1% level (t-test, two-sided).

¹ Based on mean group size.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 5

INTERGROUP COMPARISON OF MATERNAL FOOD CONSUMPTION (g/animal/day)

Period	Exposure Concentration of EEAc (ppm)				Approximate ⁱ 95% Confidence Interval
	0(control)	25	100	400	
Before exposure: (Day 0 to Day 5) Mean	97	105	104	107	±14
SD	27	19	28	33	
During exposure: (Day 5 to Day 19) Mean	90	85	78	70*	±12
SD	27	21	24	23	
After exposure: (Day 19 to Day 28) Mean	93	102	83	114**	±11
SD	23	25	24	19	
Number of females	16	15	17	19	

* Statistically significantly different from the control group mean at the 5% level (t-test, two-sided).

** Statistically significantly different from the control group mean at the 1% level (t-test, two-sided).

ⁱ Based on mean group size.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 6
MACROSCOPIC OBSERVATIONS IN THE DOES

	Exposure Concentration of EEAc (ppm)			
	0(control)	25	100	400
Thorax:				
Creamy/caseous fluid	1			
Clear fluid	1			
Abdomen:				
Creamy/caseous fluid	1			
Fat pink		1		
Lungs:				
Pleura thickened	1			
Patchy congestion	2			
Pale nodules, firm mass and patchy congestion		1		
Liver:				
Focal thickening of capsule	1			
Pale	2			
Friable	1			
Accentuated lobulation	1		3	
White granular areas			1	1
Congested	1			
Edges of lobes 'frilled'		1		
Kidneys:				
Surfaces pitted	3	2		4
Dark patches	1			
Mottled		1		
Stones and fatty material in papilla		1		
Pale lines extending from pits on surface into pelvis				1
Ovaries:				
Cystic	3	5		3
Fallopian Tubes:				
Distended and tortuous with creamy deposit		1		
Cystic		2		

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 6 - continued

MACROSCOPIC OBSERVATIONS IN THE DOES

	Exposure Concentration of EEAc (ppm)			
	0(control)	25	100	400
Uterus:				
Caseous material present and tissue reddened (cervix also reddened)			1	
Endometrium thick and convoluted		1		1
Alimentary tract:				
Contents fluid, gaseous or dark	1		4	
Total no of observations	20	16	9	10
No of animals with any observation	11	9	6	7

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 7
 INTERGROUP COMPARISON OF MATERNAL SPLEEN WEIGHTS

	Exposure Concentration of EEAc (ppm)				Approximate ¹ 95% Confidence Interval
	0(control)	25	100	400	
Spleen weight (g)Mean	0.731	0.904	0.844	0.875	±0.134
SD	0.269	0.220	0.222	0.347	
Spleen weight relative to bodyweight (%) Mean	0.031	0.038	0.035	0.035	±0.005
SD	0.012	0.010	0.010	0.012	
Number of animals	16	15	17	19	

There were no statistically significant differences from control values.

¹ Based on mean group size.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 8

INTERGROUP COMPARISON OF MATERNAL HAEMATOLOGY DATA

Parameter	Exposure Concentration of EEAc (ppm)				Approximate ¹ 95% Confidence Interval
	0(control)	25	100	400	
Haemoglobin (g/dl) Mean	12.6	12.5	12.0	11.8*	±0.5
SD	1.1	1.5	0.8	0.9	
Haematocrit Mean	0.381	0.383	0.371	0.365	±0.149
SD	0.028	0.039	0.022	0.026	
Red Blood Cell Count (x 10 ¹² /l) Mean	5.65	5.76	5.60	5.50	±0.21
SD	0.42	0.57	0.24	0.41	
Mean Cell volume (fl) Mean	68.5	67.6	67.4	67.3	±1.6
SD	3.2	3.8	3.2	2.4	
Mean Cell Haemoglobin (pg) Mean	21.8	21.3	21.0	21.0	±0.6
SD	1.9	1.1	1.0	0.8	
Mean Cell Haemoglobin Concentration(g/dl) Mean	32.5	32.1	31.8	31.8	±0.5
SD	2.0	0.9	0.6	0.4	
White Blood Cell Count (x 10 ⁹ /l) Mean	3.64	3.18	2.96	2.92	±0.91
SD	1.9	2.1	1.6	1.5	
Number of animals	13	14	16	18	

* Statistically significantly different from the control group mean at the 5% level (t-test, two-sided).

¹ Based on mean group size.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 9

INTERGROUP COMPARISON OF LITTER DATA

	Exposure Concentration of EEAc(ppm)				Approximate ⁴ 95% Confidence Interval
	0(control)	25	100	400	
No. of females mated	24	23	23	24	
No. pregnant	17	15	18	19	
No. aborted	1	0	1	0	
No. pregnant at termination	16	15	17	19	
No. with total resorptions	0	1	0	3	
No. of corpora lutea ¹					
Mean	8.5	8.2	9.2	7.2	
SD	2.2	2.5	2.8	2.8	
n	(16)	(14)	(16)	(18)	
No. of implantations					
Mean	7.3	6.3	8.1	6.5	±1.4
SD	1.8	3.4	3.3	3.0	
n	(16)	(15)	(17)	(19)	
Pre-implantation loss ¹ :					
Percentage	14.0	19.1	3.2	12.3	±0.163
Proportion of females affected	7/16	6/14	7/16	8/18	
Post-implantation loss ² :					
Percentage	9.4	13.1	6.7	24.4*	±0.164
Proportion of females affected	9/16	7/15 [17.2] [6/14]	5/15	13/19 [13.1] [10/16]	±0.125

1. Excludes three females whose corpora lutea were either not counted or were regressing and difficult to count.

2. Excludes two females which littered in the morning of their scheduled post mortem day and whose foetuses were partially eaten.

4. Based on mean group size.

[] Excludes females with total resorption.

* Statistically significantly different from the control group mean at the 5% level (t-test, one-sided, on transformed value).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 9 - continued

INTERGROUP COMPARISON OF LITTER DATA

	Exposure Concentration of EEAc (ppm)				Approximate ⁴ 95% Confidence Interval
	0(control)	25	100	400	
Intra-uterine deaths ² :					
Number early	9	9	8	24	
Percentage early	7.7	9.6	6.7	19.5	±0.164
Proportion of females affected	7/16	7/15	5/15	11/19	
		[8 8.6 6/14]		[8 7.5 8/16]	±0.110
Number late	2	8	0	6	
Percentage late	1.7	8.5	0.0	4.9	±0.086
Proportion of females affected	2/16	3/15	0/15	3/19	
No. ² of viable foetuses	Mean SD n	6.6 1.6 (16)	5.1 2.8 (15)	7.4 3.0 (15)	4.9* 3.2 (19)
		[5.5 2.5 (14)]		[5.8 2.6 (16)]	±1.3
Proportion ₃ of male foetuses		47/106	32/77	58/111	50/93
Percentage ₃ of male foetuses		44.3	41.6	52.3	53.8
Mean total ₃ litter weight	Mean SD n	230 63 (16)	194 83 (14)	223 68 (15)	179* 72 (16)
					±37
Mean live ₃ foetal weight	Mean SD n	34.7 4.9 (106)	35.2 5.9 (77)	30.1** 6.1 (111)	30.8** 4.2 (93)
					±2.2

2. Excludes two females which littered in the morning of their scheduled post mortem day and whose foetuses were partially eaten.
3. Excludes two females noted in 2 and four females with total resorption.
4. Based on mean group size.
- [] Excludes females with total resorption.
- * Statistically significantly different from the control group mean at the 5% level (t-test, one-sided).
- ** Statistically significantly different from the control group mean at the 1% level (t-test, one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 10
COMBINED EXTERNAL/VISCERAL DEFECTS

	Exposure Concentration of EEAc (ppm)				Approx 95% confidence limits
	0(control)	25	100	400	
No. litters examined	16	14	15	16	-
No. foetus examined	106	77	111	93	-
Minor Defects only					
No. fetuses affected	22	17	28	48++	-
% fetuses affected	20.8	22.1	25.2	51.6	-
Mean transformed value	0.377	0.409	0.433	0.791**	±0.189
Major Defects					
No. fetuses affected	0	1	2	3	-
% fetuses affected	0	1.3	1.8	3.2	-

1. Based on mean group size.

** Statistically significantly different from the control group mean at the 1% level (t-test, one-sided).

++ Statistically significant increase over control group proportion at the 1% level (Fisher's exact test, one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 11
SKELETAL DEFECT DATA

	Exposure Concentration of EEAc (ppm)				Approx 95% ¹ confidence limits
	0(control)	25	100	400	
No. litters examined	16	14	15	16	-
No. fetuses examined	106	77	111	93	-
<u>Variants</u>					
No. fetuses affected	50	40	72 ⁺⁺	92 ⁺⁺	-
% fetuses affected	47.2	51.9	64.9	98.9	-
Mean transformed value	0.749	0.820	0.989*	1.536**	±0.194
<u>Minor Defects only</u>					
No. fetuses affected	22	14	37 ⁺	85 ⁺⁺	-
% fetuses affected	20.8	18.2	33.3	91.4	-
Mean transformed value	0.382	0.385	0.556	1.384**	±0.163
<u>Major Defects</u>					
No. fetuses affected	1	0	0	6 ⁺	-
% fetuses affected	0.9	0.0	0.0	6.5	-
Mean <u>pes</u> score	1.47	1.42	1.72*	1.63	±0.19
Mean <u>manus</u> score	1.50	1.45	1.73*	1.92**	±0.15

1. Based on mean group size.
 * Statistically significantly different from the control group mean at the 5% level (t-test, one-sided).
 ** Statistically significantly different from the control group mean at the 1% level (t-test, one-sided).
 ++ Statistically significant increase over control group proportion at the 1% level (Fisher's exact test, one-sided).
 + Statistically significant increase over control group proportion at the 5% level (Fisher's exact test, one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 12

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : EXTERNAL/VISCERAL FINDINGS

Defect	Classification	Dose level of EEAe ppm							
		0 Air Control		25		100		400	
External Visceral		No	%	No	%	No	%	No	%
Number of foetuses examined		106		77		111		93	
Subcutaneous haemorrhage	minor	1	0.9	0	0.0	0	0.0	0	0.0
Head:									
Brain ventricles-moderate dilatation	major	0	0.0	0	0.0	0	0.0	1	1.1
Brain ventricles-slight dilatation	minor	0	0.0	0	0.0	1	0.9	0	0.0
Thorax:									
Lungs: Azygous lobe absent	minor	1	0.9	0	0.0	1	0.9	1	1.1
Abdomen:									
Left kidney: slight pelvic dilatation	minor	7	6.6	8	10.4	7	6.3	14+	15.1
Left kidney: moderate pelvic dilatation	minor	0	0.0	1	1.3	0	0.0	1	1.1
Right kidney: slight pelvic dilatation	minor	1	0.9	0	0.0	1	0.9	3	3.2
Both kidneys: slight pelvic dilatation	minor	0	0.0	2	2.6	1	0.9	7++	7.5
Both kidneys: moderate pelvic dilatation	minor	0	0.0	0	0.0	1	0.9	1	1.1
Left kidney and ureter absent									
vasculature present but no evidence of renal tissue	major	0	0.0	1	1.3	0	0.0	0	0.0
Right kidney and ureter absent									
and left kidney enlarged	major	0	0.0	0	0.0	0	0.0	1	1.1

+ Statistically significant increase compared with control group proportion, at the 5% level, (Fisher's Exact test: one-sided).

++ Statistically significant increase compared with control group proportion, at the 1% level, (Fisher's Exact test: one-sided).

TABLE 12 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : EXTERNAL/VISCERAL FINDINGS

Defect	Classification	Dose level of EEAc ppm							
		0 Air Control		25		100		400	
		No	%	No	%	No	%	No	%
Abdomen continued:									
Ureter/s dilated	minor	3	2.8	0	0.0	2	1.8	2	2.2
Gall bladder opaque/appears empty	minor	6	5.7	4	5.2	6	5.4	17++	18.3
Gall bladder reduced in size	minor	0	0.0	0	0.0	0	0.0	3	3.2
Gall bladder bilobular	minor	2	1.9	1	1.3	1	0.9	2	2.2
Gall bladder dark	minor	0	0.0	0	0.0	0	0.0	2	2.2
Spleen pale	minor	1	0.9	2	2.6	10++	9.0	13++	14.0
Spleen reduced	minor	0	0.0	3	3.9	1	0.9	13++	14.0
Secondary spleen	minor	0	0.0	0	0.0	0	0.0	1	1.1
Spleen oval	minor	0	0.0	0	0.0	0	0.0	1	1.1
Testis haemorrhagic	minor	2	1.9	0	0.0	0	0.0	0	0.0
One ovary situated higher in abdomen than other and attached to intestinal membrane	major	0	0.0	0	0.0	1	0.9	0	0.0
Blood vessels to umbilical cord surrounded by blood clot	minor	1	0.9	1	1.3	0	0.0	1	1.1
Extremities	major	0	0.0	0	0.0	1	0.9	0	0.0
Right forelimb malrotated	major	0	0.0	0	0.0	0	0.0	1	1.1
Both forelimbs malrotated (Right severe)	major	0	0.0	0	0.0	0	0.0	1	1.1

	0.0	0.05	0.1	1.1
++ Statistically significant increase compared with control group proportion, at the 1% level, (Fisher's Exact test: one-sided).				

TABLE 13

Defect	Classification	Dose level of EEAc ppm							
		0 Air Control		25		100		400	
		No	%	No	%	No	%	No	%
<u>Skeletal Defects</u>		106		77		111		93	
<u>Number of foetuses examined</u>									
<u>Skull:</u>									
Parietals partially ossified	minor	2	1.9	1	1.3	1	0.9	3	3.2
Interparietal partially ossified	minor	0	0.0	0	0.0	1	0.9	2	2.2
Interparietal not ossified	minor	1	0.9	0	0.0	0	0.0	1	1.1
Hyoid partially ossified	minor	8	7.5	3	3.9	6	5.4	10	10.8
<u>Vertebrae:</u>									
<u>Cervical vertebrae</u>									
1st centrum partially ossified	minor	1	0.9	1	1.3	10++	9.0	12++	12.9
3rd centrum partially ossified	minor	0	0.0	0	0.0	0	0.0	1	1.1
5th centrum partially ossified	minor	0	0.0	0	0.0	0	0.0	2	2.2
6th centrum partially ossified	minor	0	0.0	0	0.0	0	0.0	2	2.2
3rd centrum bipartite	minor	0	0.0	0	0.0	0	0.0	2	2.2
		0	0.0	0	0.0	0	0.0	1	1.1

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TABLE 13 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAc ppm							
		0 Air Control		25		100		400	
		No	%	No	%	No	%	No	%
Vertebrae continued:									
Cervical Vertebrae continued:									
1st centrum not ossified	minor	0	0.0	1	1.3	1	0.9	4+	4.3
3rd centrum not ossified	minor	0	0.0	0	0.0	0	0.0	1	1.1
4th centrum not ossified	minor	0	0.0	0	0.0	0	0.0	1	1.1
3rd arch partially ossified	minor	0	0.0	0	0.0	0	0.0	0	0.0
Transverse processes of 7th cervical vertebra partially ossified	minor	0	0.0	0	0.0	1	0.9	0	0.0
Thoracic vertebrae	minor	0	0.0	1	1.3	0	0.0	0	0.0
7th centrum partially ossified	minor	0	0.0	0	0.0	0	0.0	1	1.1
Lumbar vertebrae									
3rd transverse process partially ossified	minor	0	0.0	0	0.0	0	0.0	0	0.0
4th-7th transverse processes partially ossified	minor	0	0.0	0	0.0	1	0.9	0	0.0
Sacral vertebrae	minor	0	0.0	0	0.0	0	0.0	1	1.1
1st transverse processes partially ossified	minor	0	0.0	0	0.0	0	0.0	0	0.0
Caudal vertebrae									
Misaligned	minor	0	0.0	0	0.0	0	0.0	1	1.1
25 presacral vertebrae	minor	0	0.0	0	0.0	0	0.0	1	1.1
27 presacral vertebrae	minor	1	0.9	0	0.0	0	0.0	0	0.0
	minor	7	6.6	1	1.3	11	9.9	85++	91.4

+ Statistically significant increase compared with control

	1.3	11	9.9	85++	91.7
+	Statistically significant increase compared with control group proportion, at the 5% level (Fisher's test: one-sided).				

++ Statistically significant increase compared with control group proportion, at the 5% level (Fisher's Exact test: one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 13 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAe ppm							
		0 Air Control		25		100		400	
		No	%	No	%	No	%	No	%
Vertebrae continued: <u>Multiple Defects</u> 1st right cervical arch not ossified. Enlarged arch beside 2nd and 3rd cervical centra and therefore either 2nd or 3rd arch not ossified. Extra centre of ossification between 4th and 5th thoracic vertebrae. 4th thoracic centrum bipartite 4th rib right side split	major	1	0.9	0	0.0	0	0.0	0	0.0
5th caudal partially ossified 6th caudal bipartite. 12th and 13th caudals fused.	minor	0	0.0	0	0.0	1	0.9	0	0.0
An extra half of a vertebra between 2nd and 3rd lumbar vertebrae. 1st-4th sacral vertebral arches misaligned 2nd-4th sacral vertebrae partially ossified. 1st-2nd caudal vertebrae partially ossified. 1st caudal vertebral arches misaligned	major	0	0.0	0	0.0	0	0.0	1	1.1

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 13 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAe ppm					
		0 Air Control		25		100	
		No	%	No	%	No	%
Vertebrae continued: 2nd sacral vertebra partially ossified 1st-3rd sacral vertebrae misaligned. 6th and 8th caudal vertebrae bipartite and partially ossified. 12th-17th caudal vertebrae mishapen and misaligned.	major	0	0.0	0	0.0	0	0.0
						1	1.1
10th, 11th and 12th thoracic centra misaligned. 11th thoracic arch right side not ossified. 11th and 12th ribs on right side arising from the same vertebra.	major	0	0.0	0	0.0	0	0.0
						1	1.1
9th thoracic vertebra-right arch not ossified. Thoracic vertebrae after the 9th slightly misaligned. 1st and 2nd lumbar vertebrae misaligned and partially fused. Ribs 8 and 9 on right side fused along one-third of their length	major	0	0.0	0	0.0	0	0.0
						1	1.1

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 13 - continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAe ppm					
		0 Air Control		25		100	
		No	%	No	%	No	%
Vertebrae continued: 10th thoracic vertebra-left arch partially ossified resulting in misalignment of 11th and 12th thoracic vertebrae and also 1st lumbar vertebra. 1st lumbar centrum partially ossified.	major	0	0.0	0	0.0	0	0.0
						1	1.1
3rd - 5th cervical centra partially ossified and misaligned	major	0	0.0	0	0.0	0	0.0
						1	1.1
Ribs: 13 unilateral 13 bilateral 14 unilateral (short) Extra cervical rib unilateral and short Rib anomalies 3rd rib left side split	variant	11	10.4	9	11.7	8	7.2
	variant minor	24 0	22.6 0.0	14 0	18.2 0.0	56++ 0	50.5 0.0
	minor	0	0.0	0	0.0	0	0.0
	minor	0	0.0	0	0.0	0	0.0
						1	1.1
						89++ 2	95.7 2.2
						1	1.1
						1	1.1

++ Statistically significant increase compared with control group proportion, at the 1% level, (Fisher's Exact test: one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

TABLE 13 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAe ppm							
		0 Air Control		25		100		400	
		No	%	No	%	No	%	No	%
Sternebrae:									
1st sternebra partially ossified	minor	0	0.0	0	0.0	1	0.9	2	2.2
2nd sternebra partially ossified	minor	1	0.9	2	2.6	7+	6.3	29++	31.2
3rd sternebra partially ossified	minor	0	0.0	1	1.3	0	0.0	1	1.1
4th sternebra partially ossified	minor	0	0.0	0	0.0	0	0.0	3	3.2
5th sternebra partially ossified	variant	25	23.6	20	26.0	20	18.0	39++	41.9
6th sternebra partially ossified	minor	0	0.0	1	1.3	0	0.0	16++	17.2
5th sternebra not ossified	minor	1	0.9	1	1.3	3	2.7	3	3.2
6th sternebra not ossified	minor	0	0.0	0	0.0	1	0.9	3	3.2
2nd sternebra bipartite	minor	0	0.0	1	1.3	0	0.0	8++	8.6
5th sternebra bipartite	minor	1	0.9	0	0.0	0	0.0	5	5.4
6th sternebra bipartite	minor	0	0.0	0	0.0	0	0.0	1	1.1
1st sternebra misaligned	minor	0	0.0	0	0.0	0	0.0	2	2.2
2nd sternebra misaligned	minor	0	0.0	0	0.0	0	0.0	2	2.2
3rd sternebra misaligned	minor	0	0.0	0	0.0	0	0.0	3	3.2
4th sternebra misaligned	minor	0	0.0	0	0.0	0	0.0	2	2.2
5th sternebra misaligned	minor	0	0.0	0	0.0	0	0.0	1	1.1
Extra centre of ossification above 1st sternebra	minor	0	0.0	4+	5.2	1	0.9	0	0.0

+ Statistically significant increase compared with control group proportion, at the 5% level, (Fisher's Exact test: one-sided).

++ Statistically significant increase compared with control group proportion, at the 1% level, (Fisher's Exact test: one-sided).

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

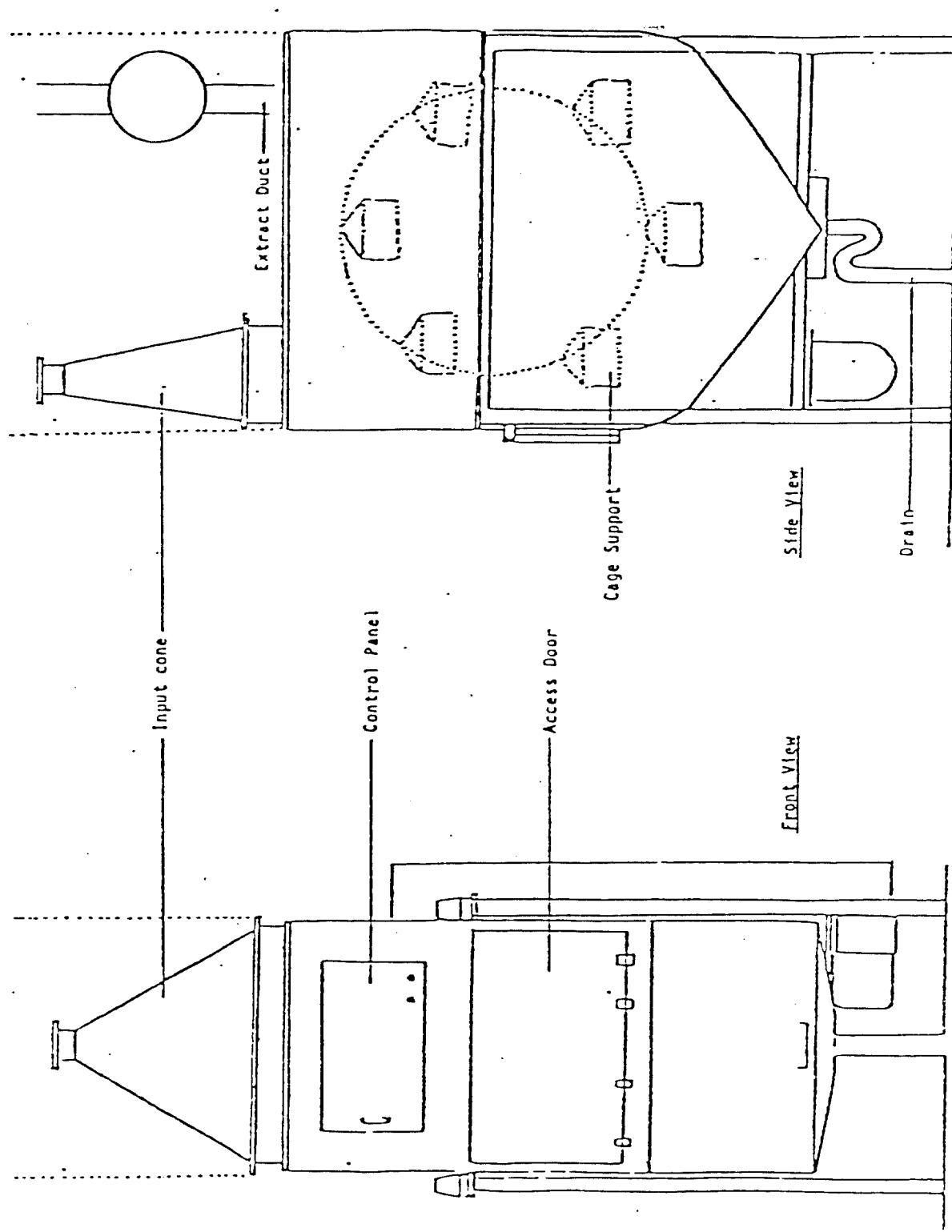
TABLE 13 - continued

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE : SKELETAL FINDINGS

Defect	Classification	Dose level of EEAe ppm					
		0 Air Control		25		100	
		No	%	No	%	No	%
Sternebrae continued: Extra centre of ossification between 2nd and 3rd sternbrae Extra centre of ossification between 5th and 6th sternbrae Sternebrae 4 and 5 fused 6th sternbrae abnormal shape	minor	0	0.0	0	0.0	0	0.0
	minor	3	2.8	0	0.0	5	4.5
	minor	1	0.9	0	0.0	0	0.0
	minor	0	0.0	0	0.0	0	0.0
Pelvic Girdle: Pubes partially ossified	minor	1	0.9	0	0.0	0	0.0
						1	1.1
						5	5.4
						0	0.0
						1	1.1
						5	5.4

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

FIGURE 1



ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 1

ANALYTICAL DETAILS OF THE TEST MATERIAL USED IN THE STUDY

Description	Clear, free from suspended matter
Colour (Hazen Units)	<5
Water content (% w/w)	0.02
Ester content (% w/w)	99
Acidity (% w/w as acetic acid)	0.010
Specific gravity (20/20°C)	0.975
Residue on evaporation (% w/w)	<0.001
Distillation range °C	
Initial boiling point	155.2
Dry point	159.3

Confirmation of identity was by comparison with a reference infra-red spectrum of EEAc.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 2

CHEMICAL COMPOSITION OF LABORATORY ANIMAL DIETS (the vitamin and trace mineral composition of pelleted diets refers to the amounts of each nutrient added to the diet and ignores the natural sources).

Diet CRB (Rabbit Breeder)

Calculated Analysis

Crude Oil	
Crude Protein	1.9%
Crude Fibre	16.1%
Calcium (as Ca)	14.0%
Phosphorus (as P)	1.1%
Salt (as NaCl)	0.6%
Metaboliseable Energy	0.7%
	1934 Kcal/kg

Trace Elements Added

Manganese	
Copper	125ppm
Cobalt	7ppm
Iron	0.4ppm
Iodine	30ppm
Magnesium	1.3ppm
	102ppm

Vitamins Added (per kg)

Vitamin A	
D ₃	8000IU
B ₂	1000IU
Nicotinic Acid	8mg
Pantothenic Acid	50mg
	12mg

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 2 - continued

Vitamins Added (per kg)

Vitamin B ₁₂	12µg
E	60I.U.
K	10mg
Folic Acid	10mg
Choline chloride	200mg
Vitamin B ₁	4mg
Vitamin B ₆	6mg

Amino Acids (as percentage of feed)

Threonine	0.6
Glycine	0.8
Valine	0.8
Cystine	0.2
Methionine	0.2
Isoleucine	0.7
Leucine	1.2
Tyrosine	0.6
Phenylalanine	0.7
Lysine	0.9
Histidine	0.4
Arginine	1.1
Tryptophan	0.1

An analysis of each batch of diet for major constituents and contaminants was supplied by CRB. This was checked for acceptability, based on the best available information at the time, before the batch was used on the study. The known contaminants found in the diet were not considered to be present in sufficient concentration to have had an influence on the outcome of the study.

Water

Analyses of tap water were carried out periodically and checked for acceptability. The known contaminants found in the water were not considered to be present in sufficient concentration to have had an influence on the outcome of the study.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 3

TEMPERATURE AND RELATIVE HUMIDITY IN ANIMAL HOLDING
AREA AND EXPOSURE CHAMBERS

	Temperature ¹ °C	Range	Relative ¹ Humidity %	Range
Holding area	23.1 (47) ± 1.3	20.5-26.5	61.4 (46) ± 6.2	47-76
Exposure chambers:				
0ppm EEAc	24.8 (23) ± 1.0	23.25-26.75	60.3 (23) ± 4.5	53-70
25ppm EEAc	25.0 (23) ± 1.0	23.5 -27.0	61.7 (22) ± 3.6	53-68
100ppm EEAc	25.2 (23) ± 1.0	23.5 -27.0	58.7 (22) ± 4.1	52-67
400ppm EEAc	25.7 (23) ± 0.9	24.25-27.5	56.4 (22) ± 4.5	47-66

¹ Values are means ± standard deviation with numbers of readings in parentheses.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
 INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 4

ALLOCATION OF MATED DOES TO GROUP

Day of Mating	Week 1 of Mating				Week 2 of Mating			
	1	2	3	4	1	2	3	4
Group Nos	1	2	3	4	1	2	3	4
	2	3	4	1	2	3	4	1
	3	4	4	2	3	4	1	2
	4	1	1	3	4	1	2	3
	1	2	2	4	1	2	3	4
	2	3	3	1	2	3	4	1
	3	4	1	2	3	4	1	2
	4	1	2	3	4	1	2	3
	1	2	3	4	1	2	3	4
	2	3	4	1	2	3	4	1
	3	4	1	2	3	4	1	2
	4	1	2	3	4	1	2	3

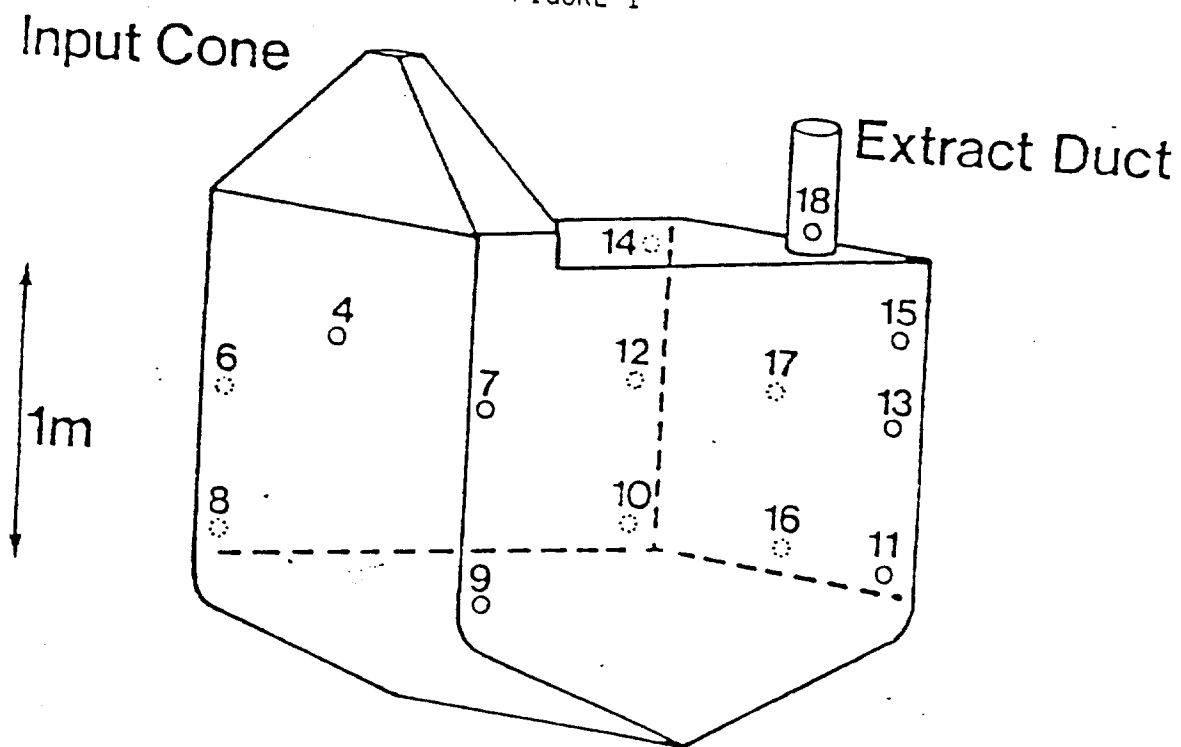
ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 5

The Concentration - Time Relationship and Distribution of EEA_c in 3.4m³ Exposure Chamber.

The concentration of EEA_c was analysed from several points around the chamber using an infra-red analyser (MIRAN) set to pathlength 8.25m, wavelength 8.9µm, slit width 1mm. The positions of the sampling point are indicated in figure 1.

FIGURE 1



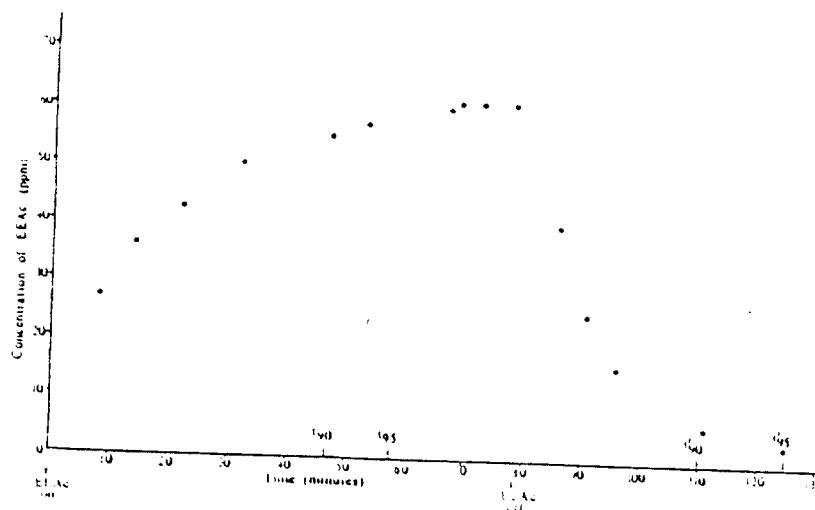
The flow rate of EEA_c was set to produce a nominal chamber concentration of approximately 60ppm at a chamber air flow rate of 600 L/min. The concentration of EEA_c at point 17 was monitored.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 5 - continued

The time to reach 90 and 95% (t_{90} , t_{95}) of the nominal concentration was 47 and 58 minutes respectively; the time to clear the chamber by 90 and 95% (t'_{90} , t'_{95}) was 32 and 46 minutes respectively. These results are shown in figure 2.

FIGURE 2



ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 5 - continued

In two separate runs, with two different nominal concentrations, several points were compared with point 17. The results are shown in Table 1 and demonstrate that all the points were within 5% of the mean concentration with the exception of point 4 which was within 7% of the mean.

Table 1

	Point No.	Concentration of EEAc (ppm)
Run 1	17	68
	7	68
	9	69
	11	71
	17	71
	13	72
	15	69
	16	73
	17	<u>73</u>
	mean	70.4
	S.D.	2.0
Run 2	Point No.	(ppm)
	17	98
	4	105
	8	97
	6	98
	10	98
	12	97
	14	93
	18	95
	17	<u>102</u>
	mean	98.1
	S.D.	3.6

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

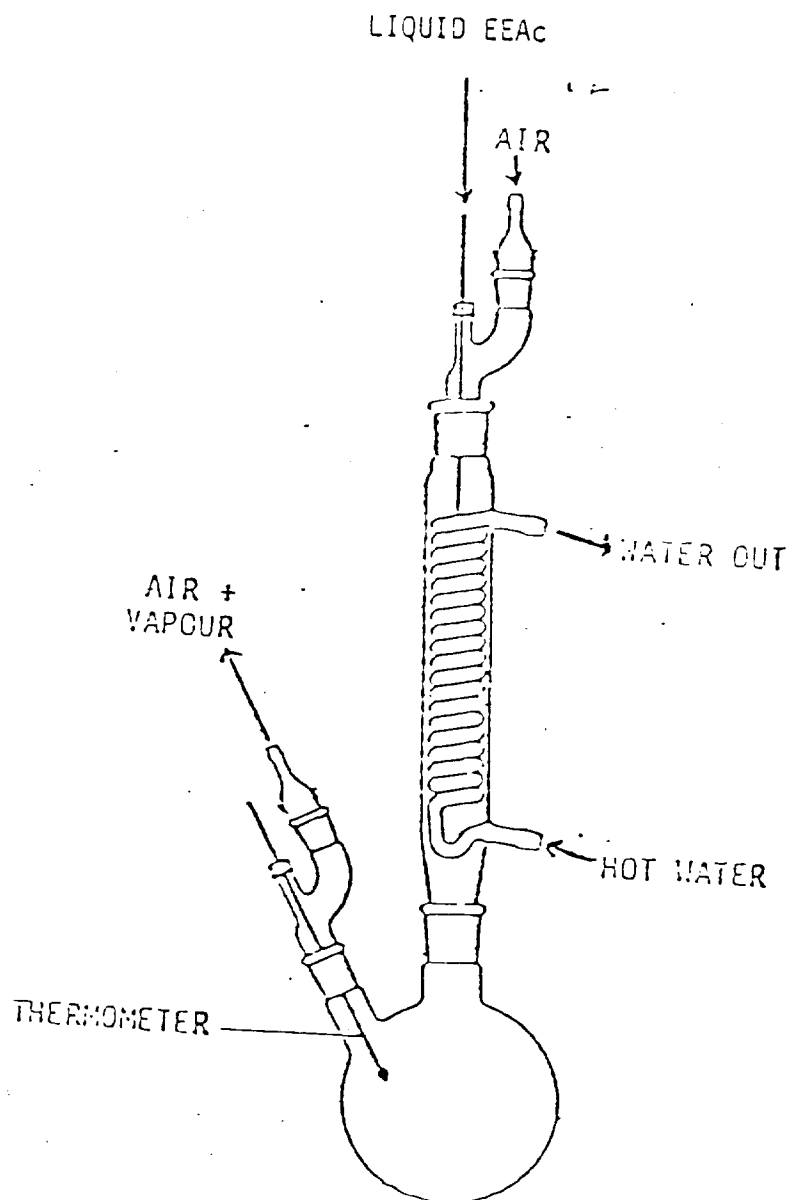
APPENDIX 5 - continued

The data demonstrate the validity of using point 17 as the atmosphere sampling point in the EEAc studies and the uniformity of distribution of EEAc in these chambers.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 6

METHOD OF ATMOSPHERE GENERATION FOR EEAc



The generation system for each exposure level consisted of a reservoir of EEAc, a peristaltic pump (GILSON) a glass condenser, and a round-bottom flask.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 6 - continued

METHOD OF ATMOSPHERE GENERATION FOR EEAc

The EEAc was metered from the reservoir onto the condenser coil by means of the peristaltic pump fitted with solvent resistant tubing (ISOVERSINIC, GILSON). Hot water 35-40°C from a thermocirculator (CHURCHILL) was circulated through the condenser coil to aid in the volatilisation of the EEAc.

A carrier air flow of 2-6l/min was passed down the condenser jacket and through the flask. The carrier air plus the EEAc vapour was then passed into the input air of the chamber.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 7

The Determination of Ethylene Glycol Monoethyl Ether Acetate (EEAc) in Test Atmospheres.

Method Summary

The test atmosphere was drawn from the chamber into a MIRAN infra-red analyser using a small pump. The absorbance given by the test atmosphere was compared to a calibration curve to calculate the concentration of EEAe.

Calibration Standards

1.2749g of EEAe was weighed into a 100ml standard flask and diluted to the mark with carbon disulphide.

Procedure

(i) Calibration

(a) Control and 25ppm levels

Aliquots of the above calibration standard were cumulatively injected into a closed loop incorporating the MIRAN gas cell and a small pump. The absorbance obtained was read off the meter and a calibration curve was drawn up of concentration (ppm) vs absorbance.

(b) 100 and 400ppm levels

Procedure as in (a) but cumulative aliquots of neat EEAe were injected into the closed loop.

(ii) Sampling

The test atmosphere was drawn from a point drilled in the back wall of the exposure chamber to the MIRAN infra-red analyser via PTFE tubing (4mm id) using a small vacuum pump.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 7 - continued

(iii) Infra Red conditions

The test was carried out using MIRAN 104 infra red analysers with variable pathlength gas cell fitted.

Typical conditions were:-

Wavelength: 9.2 μ m
Slit width: 1mm
Range: 1 AUFS (100 and 400ppm level)
: 0.25 AUFS (control and 25ppm levels)
Pathlength: 8.5m (100 and 400ppm levels)
: 14.5m (control and 25ppm levels)
Meter Response: 4 (100 and 400ppm levels)
10 (control and 25ppm levels)

(iv) Calculation of results

The concentration of the test atmosphere was read directly from the calibration curve.

(v) Limit of Determination

The limit of determination was set at 0.25ppm.

Note:- Calculation of concentrations of EEAc in closed loop system

$$\text{Concn. (ppm)} = \frac{A \times V_m}{V_L \times M}$$

A = mg EEAc injected into loop

V_L = volume of closed loop (2.54 l)

V_m = molar gas volume 24400 at 20°C

M = molecular weight EEAc (132.2)

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

APPENDIX 8

SCALE FOR ASSESSMENT OF SKELETAL OSSIFICATION OF THE
MANUS AND PES OF RABBITS

Scale

- 1 (good) Metacarpals/metatarsals and 1st, 2nd and 3rd rows of phalanges fully ossified.
- 2 Metacarpals/metatarsals and 1st and 3rd rows of phalanges fully ossified, some of 2nd row not ossified.
- 3 Metacarpals/metatarsals fully ossified. All 1st and 3rd row present, the majority being fully ossified, most of 2nd row not ossified although occasional phalanx may be partially ossified.
- 4 One metacarpal or metatarsal may be partially ossified, remainder of the metatarsals or metacarpals fully ossified. Second row of phalanges not ossified, most of 1st and 3rd rows of phalanges fully ossified but a few partially ossified.
- 5 (poor) One metacarpal or metatarsal partially ossified or not ossified, remainder of metatarsals and metacarpals fully ossified. Second row of phalanges not ossified, occasional phalanges in 1st and 3rd row not ossified, remainder partially ossified.

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE (EEAc):
INHALATION TERATOGENICITY STUDY IN RABBITS

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 30 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

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U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan
Terry R. O'Bryan
Risk Analysis Branch

Enclosure

12456A



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

Triage of 8(e) Submissions

Date sent to triage: _____

NON-CAP

CAP

Submission number: 12456A

TSCA Inventory:

Y

N

D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

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w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

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entire document: 0

1

2

pages 1,2

pages 1,2,4,6

Notes:

Contractor reviewer : LPS

Date: 2/16/95

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:

Submission # 8EHQ-0992-12456 SEQ. A

TYPE: INT SUPP FLWP

SUBMITTER NAME: Union Carbide Corporation

INFORMATION REQUESTED: FLWP DATE:

0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:

0632 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:

0401 NO ACTION REPORTED
0402 STUDIES PLANNED/IN PROGRESS
0403 NOTIFICATION OF WORK RATIONALE
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 09/24/92 OTS DATE: 09/29/92 CSRAD DATE: 02/09/95

CHEMICAL NAME:

CAS#

111-15-9

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPI/CLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	<u>0242</u> IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	<u>0243</u> CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
<u>0206</u> REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCC/REL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
<u>0207</u> REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0299 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0239 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

TRIAGE DATA: NON-CBI INVENTORY

YES

ONGOING REVIEW

YES (DROP/REFER)

SPECIES

RBT

TOXICOLOGICAL CONCERN:

LOW

MED

HIGH

USE:

PRODUCTION:

CAS SR

NO

NO (CONTINUE)

IN HUMANI

REFER

099212 inhalation, development

400 ppm - reduced dam gain, reduced blood hemoglobin levels
inc total resorptions + reduced fetal wt
retarded ossification, major malformations of
ventral column
100 ppm - reduction in fetal wt, retarded ossification
25 ppm - NOAEL for developmental toxicity.